ESTIMATION OF METFORMIN HYDROCHLORIDE AND VOGLIBOSE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

A dissertation submitted to

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MASTER OF PHARMACY

IN

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SUBMITTED

BY

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CERTIFICATE

This is to certify that the dissertation entitled **"ESTIMATION OF METFORMIN HYDROCHLORIDE AND VOGLIBOSE IN TABLET DOSAGE FORM BY RP-HPLC METHOD"** submitted by **M.S.HARIKRISHNAN** (Reg No: 261330954) in partial fulfillment for the award of degree of Master of Pharmacy to the **Tamilnadu Dr. M.G.R Medical University, Chennai** is an independent bonafide work of the candidate carried out under my guidance in**,** Department of Pharmaceutical Analysis, Edayathangudy.G.S Pillay College of Pharmacy during the academic year 2015-2016.

Place: Nagapattinam **Prof.Dr.D.Babu Ananth, M.Pharm., Ph.D.,**

Date:

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INTRODUCTION

Analytical chemistry is a branch of chemistry that Ideals with the separation, identification and determination of components in a sample. It is the science of making quantitative measurements, which requires background knowledge of chemical and physical concepts. Analytical chemistry may be defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained".

Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches of science like Chemistry, Physics, Microbiology, Nuclear Science, Electronics, etc. Analytical method is a specific application of a technique to solve an analytical problem. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air

Pharmaceutical analysis techniques are applied mainly in two areas

Traditionally, analytical chemistry has been split into two main types, qualitative and quantitative:

1. Qualitative

Qualitative analysis is to establish the presence of a given element or compound in a sample.

2. Quantitative

Quantitative analysis is to establish the amount of a given element or compound in a sample.

Specific Technologies and Instrumentation

A) Spectrometric techniques

- Ultraviolet and visible Spectrophotometry
- Fluorescence and phosphorescence Spectrophotometry
- Atomic Spectrometry (emission and absorption)
- Infrared Spectrophotometry
- Raman Spectroscopy
- X-Ray Spectroscopy
- Radiochemical Techniques including activation analysis
- Nuclear Magnetic Resonance Spectroscopy
- Electron Spin Resonance Spectroscopy

B) Electrochemical Techniques

- Potentiometry
- Voltametry
- Voltametric Techniques
- Amperometric Techniques
- Colorimetry
- Electrogravimetry
- Conductance Techniques

C) Chromatographic Techniques

- Gas Chromatography
- High performance Liquid Chromatography
- Thin Layer Chromatography

D) Miscellaneous Techniques

- Thermal Analysis

- Mass Spectrometry
- Kinetic Techniques

E) Hyphenated Techniques

- GC-MS (Gas Chromatography – Mass Spectrometry)
- ICP-MS (Inductivity Coupled Plasma Mass Spectrometry)
- GC-IR (Gas Chromatography – Infrared Spectroscopy)
- MS-MS (Mass Spectrometry – Mass Spectrometry)

ANALYTICAL METHOD DEVELOPMENT

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-Pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit / demerits are made available

Steps of method development:

Documentation starts at the very beginning of the development process, a system for full documentation of the development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database.

1. Analyte standard characterization

All known information about the analyte and its structure is collected i.e., physical and chemical properties.

The standard analyte (≈100% purity) is obtained. Necessary arrangement is made for the proper storage (refrigerator, desicators, freezer). When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for

each one is determined. Only those methods (spectroscopic, MS, GC, HPLC etc.,) that are compatible with sample stability are considered.

2. Method requirements

The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, selectivity, linearity, range, accuracy and precision are defined.

3. Literature search and prior methodology

The literature for all types of information related to the analyte is surveyed. For synthesis, physical and chemical properties, solubility and relevant analytical methods.Books, periodicals, chemical manufacturers and regulatory agency compendia such as USP / NF, AOAC and ASTM publications are reviewed. Chemical Abstracts Service (CAS) automated computerized literature searches are convenient.

4. Choosing a method

Using the information in the literatures and prints, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analytes and samples.

If there are no prior methods for the analyte in the literature, from analogy, the compounds that are similar in structure and chemical properties are investigated and are worked out. There is usually one compound for which analytical method already exist that is similar to the analyte of interest.

5. Instrumental setup and initial studies

The required instrumentation is setup. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified.

Always new consumables (e.g. solvents, filters and gases) are used, for example, method development is never started, on a HPLC column that has been used earlier.

The analyte standard in a suitable injection / introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (e.g., bulk drug), then it is possible to start work with the actual sample.

6. Optimization

During optimization one parameter is changed at a time, and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan, and every step is documented (in a lab notebook) in case of dead ends.

7. Documentation of analytical figures of merit

The originally determined analytical figures of merit limit of quantificatation (LOQ), Limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc., are documented.

8. Evaluation of method development with actual samples

The sample solution should lead to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.

9. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

Percent recovery of spiked, authentic standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average +/- standard deviation) from sample to sample and whether recovery has been optimized has been shown. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainty.

The validity of analytical method can be verified only by laboratory studies. Therefore documentation of the successful completion of such study is a basic requirement for determining whether a method is suitable for its intended applications.

INTRODUCTION TO VALIDATION

Validation

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications

Validation is defined as follows by different agencies

Food and Drug administration (FDA)

Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO)

Action of providing that any procedure, process, equipment, material, activity, or system actually leads to the expected results

European Committee (EC)

Action of providing in accordance to the principles of good manufacturing practice that any procedure, process, equipment, material, activity or system actually leads to the expected results. In brief validation is a key process for effective Quality Assurance.

Reasons for Validation

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an

integral part of the quality-control system. The second is that current good manufacturing practice regulation requires assay validation.

Steps followed for validation procedures

- Proposed protocols or parameters for validations are established.
- Experimental studies are conducted.
- Analytical results are evaluated.
- Statistical evaluation is carried out.
- Report is prepared documenting all the results.

Objective and Parameters of Analytical Method Validation

The purpose of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH guidelines, typical analytical performance characteristics that should be considered in the validation of the types of methods are

1. Accuracy
2. Precision
3. Specificity
4. Detection Limit
5. Quantification Limit
6. Linearity
7. Range
8. Ruggedness
9. Robustness

1. Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. The ICH documents recommended that accuracy should be assessed

using a minimum of nine determinations over a minimum of three concentrations levels the specified range (i.e, three concentrations and three replicates of each concentration)

Accuracy was tested (% Recovery and % RSD of individual measurements) by analyzing samples at least in triplicate, at each level (80,100 and 120 % of label claim) is recommended. For each determination fresh samples were prepared and assay value is calculated. Recovery was calculated from regression equation obtained in linearity study. Accuracy was determined from the mean relative error for a set of replicate analysis (i.e. the difference between measured and nominal concentration) for spiked samples.

2. Precision

The precision of an analytical procedure expresses the closeness of agreement between the series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variations of a series of measurements. The ICH documents recommend the repeatability should be assessed using a minimum of nine determinations covering specified range of procedure. Precision may be the measure of either the degree of reproducibility or repeatability of the analytical method under normal operating conditions.

Repeatability:

Repeatability expresses the precision under the same operating condition over a short interval of time. Repeatability is also termed intra – day assay precision.

Intermediate Precision: Intermediate precision expresses with in laboratories variations: different days, different analyst and different equipment.

Reproducibility:

When the procedure is carried out by different analyst in different laboratories using different equipment, regents and laboratories setting. Reproducibility was determined by measuring repeatability and the intermediate precision. Reproducibility is assayed by means of an inter-laboratory trial.

3. Specificity

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities.

An ICH document defines specificity as the ability to assess unequivocally the analyte in the presence compounds that may be expected to products and matrix components.

The definition has the following implications:

Identification test: Suitable identification tests should be able to discriminate compounds of closely related structure which are likely to be present .Ensure identity of an analyte .The analyte should have no interference from other extraneous components and be well resolved from them.

Purity Test: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurity of the content of impurity of an analyte i.e. related substances test, heavy metals, residual solvents etc.

Assay: To provide an exact result, this allows an accurate statement on the content or potency of the analyte in a sample.

Detection Limit

It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities as an exact value, under the stated experimental conditions. The detection limit is usually expressed as the concentration of analyte (percentage parts per million) in the sample.

4.Determination of detection limit

For instrumental and non- instrumental methods detection limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected.

LOD = 3 * SD / slope of calibration curve

SD = Standard deviation of intercepts

4.Quantification Limit (QL)

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Quantification limit is expressed as the concentration of analyte (e.g- % ppm) in the sample.

Determination of quantification limit

For instrumental and non- instrumental methods, the quantitation limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision.

LOQ = 10 * SD / slope of calibration curve

SD = Standard deviation of intercepts

Based on Standard Deviation of the blank

Measurement of the magnitude of the analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

Based on the calibration curve

A specific calibration curve should be studied using the samples, containing an analyte in the range of QL. The residuals SD of regression line or the S.D of intercepts of regression lines may be used as the S.D. The quantitative limit is a parameter of quantitative assay for low levels of compounds in sample matrices, and is use particularly for the determination of impurities or degradation products.

6. Linearity and Range

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to concentration of analyte in samples. The range of an analytical is the intervals between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated which it has been demonstrated that the analytical procedure has a suitable level of precision accuracy and linearity.

Determination of linearity and range

The linearity and range determined by application of the procedure to a series of Samples having analyte concentration spanning the claimed range of procedure. When the relationship between response and concentration is not linear, standardization may be providing by means of a calibration curve. The ICH recommends that for the establishment of linearity a minimum of five concentrations normally used.

7. Ruggedness

Degree of reproducibility (Ruggedness) of test results obtained by the analysis of the same samples under a variety of condition such as different laboratories, different analysts, different instruments etc, normally expressed as the lack of influence on test results of operational and environmental variable of the analytical method.

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. Degree of representative of test results is then determined as a function of the assay variable.

By analysis of aliquots from homogenous lots in different laboratories, by different analyst, using operational and environmental conditions that may differs but are still with in the specified parameter of the assay variable.

8. Robustness

Robustness of an analytical method is measure of its capacity to remain unaffected small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Testing varying some or all condition:

-Column temperature

-PH of buffer in mobile phase

-Reagents and flow rate

-Mobile Phase Changes

8. System Suitability

System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole.

According to USP system suitability are an integral part of chromatographic methods. These tests verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. One consequence of the evaluation of robustness and ruggedness should be that a series of system suitability parameters is established to ensure that the validity of the analytical method is maintained whenever used.

The parameters that are affected by the changes in chromatographic conditions are,

- Capacity factor (k'),
- Peak asymmetry / tailing factor (As)
- Column efficiency (N) and Selectivity (α)
- Capacity factor (k')

$k' = (t_R - t_0) / t_0$

The capacity factor is a measure of the degree of retention of an analyte relative to an unretained peak, where t_R is the retention time for the sample peak and t_o is the retention time for an unretained peak.

Recommendations

The peak should be well-resolved from the other peaks and the void volume. Generally the value of k' is > 2.

Tailing factor (T)

A measure of the symmetry of a peak, given by the following equation where $W_{0.05}$ is the peak width at 5% height and f is the distance from peak front to

apex point at 5% height. Ideally, peaks should be Gaussian in shape or totally symmetrical.

$T = W_{0.05} / 2f$

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak. Integrator variables are preset by the analyst for optimum calculation of the area for the peak of interest.

Recommendations

T of </= 2

Theoretical plate number / Efficiency (N)

A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry. The most common are shown here, with the ones most sensitive to peak shape shown first:

4-sigma / tangential

$N = 16 (t_R / W_{) 2} = L / H$

Half height

$N = 5.54 (t_R / W)^2 = L / H$

Theoretical plate number is a measure of column efficiency,

Theoretical plate number is a measure of column efficiency, that is, how many peaks can be located per unit run-time of the chromatogram, where t_R is the retention time for the sample peak and W is the peak width.

N is moderately constant for each peak on a chromatogram with a fixed set of operating conditions. H, or HETP, the Height Equivalent of a Theoretical Plate,

measures the column efficiency per unit length (L) of the column. Parameters which can affect N or H include

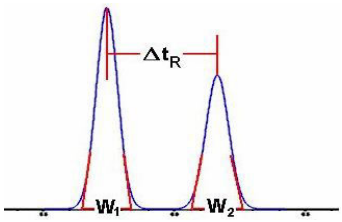
Peak position, particle size in column, flow-rate of mobile phase, column temperature, viscosity of mobile phase, and molecular weight of the analyte

Recommendations

The theoretical plate number depends on elution time but in general should be > 2000.

Resolution (R_s)

Ability of a column to separate chromatographic peaks, Resolution can be improved by increasing column length, decreasing particle size, increasing temperature, changing the eluent or stationary phase. It can also be expressed in terms of the separation of the apex of two peaks divided by the tangential width average of the peaks.



$R_s = \Delta t_R / W_1/2 + W_2/2;$

Where $\Delta t_{R =} t_2 - t_1$

For reliable quantitation, of well-separated peaks are essential for quantitation. Recommendations

Rs of > 2 between the two peaks of interest and the closest potential interfering peak (impurity, excipient, degradation product, internal standard, etc.) are desirable.

Statistical Analysis

Statistical procedures and representative calculations

The consistency and suitability of the developed method are substantiated through the statistical analysis like standard deviation, relative standard deviation and theoretical plates per meter.

For Accuracy: Standard deviation = $\sigma = \sqrt{\frac{\sum (x - x_i)^2}{n-1}}$

Where, x = sample, x_i = mean value of samples, n = number of samples

Relative Standard Deviation = $\sigma/x_{i \times 100}$

Molar extinction coefficient (mol^{-1} cm^{-1}) = A/C × L

Where, A= Absorbance of drug, C= concentration of drug, L= Path length

Sandell, s sensitivity ($\mu g/cm^2$/0.001 absorbance units) = C/A×0.001

Where, C= concentration of drug, A= Absorbance of drug

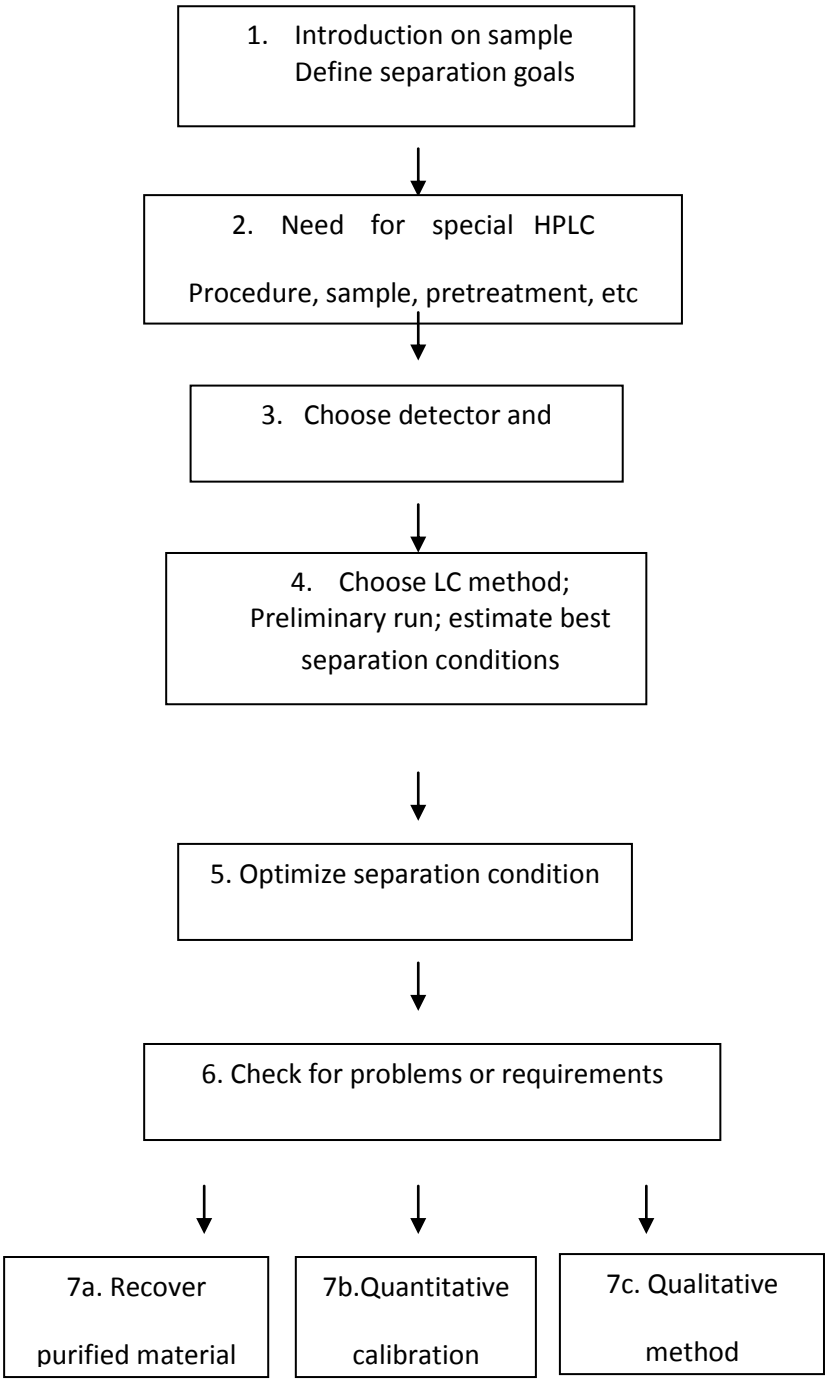
Selection of Internal Standard

A compound added to a sample in known concentration to facilitate the qualitative identification and/or quantitative determination of the sample components. Internal standards (IS) - substance used as reference in quantitative analysis; the internal standard is first mixed with standard solutions; later it is added to the unknown, and the ratio of peak heights (or areas) of internal standard and analyte is used for quantitative analysis.

HPLC method validation

Everyday many chromatographers face the need to develop a HPLC separation whereas individual approaches may exhibit considerable diversity; method development often follows the series of steps summarized as:

Fig .1 Steps involved in HPLC method validation[1]



AIM AND SCOPE OF THE WORK

1. An RP-HPLC method for the Simultaneous estimation of Metformin Hcl and Voglibose tablets was developed and Validated in Micro labs in Hosur.
2. The scope of the study is to Development and validation of Metformin HCl and Voglibose (500mg&0.3mg) using parameters like system precision and system suitability, specificity, precision, accuracy, linearity, robustness.
3. The method has been validated as per the guidelines given by ICH requirements to assure that the method consistently meets the predetermined specifications and quality attributes.

PLAN OF WORK

Literature survey

Through survey of literature available for Metformin HCl and Voglibose, regarding their physical and chemical properties, pharmacology, pharmacokinetics and reported analytical methods, forms the basis for the development of new RP-HPLC method for simultaneous analysis of these drugs were designed.

Procurement of samples

Procurement of the drug specimens draws utmosts priority. Both the drugs obtained from Micro Laboratory as gift samples and characterized by their melting points.

Development of sample

1. Selection of solvent system.

2. Selection of mobile phase.

3. Simultaneous method development for assay

4. Analysis of the commercially available formulations.

Analytical validations of developed method according to ICH guideline parameters, which are selected for method validation, are as follows.

1. Specificity

2. Selectivity

3. Precision

a. Repeatability

b. Intermediate precision

4. Linearity

5. Accuracy

6. Robustness

7. Stability of analytical solutions

VALIDATION PARAMETERS

Prepare the mobile phase and arrange all the parameters as per optimized method.

I. SYSTEM PRECISION SYSTEM SUITABILITY

I.a. SYSTEM PRECISION

Preparation of standard solution:

Solution-A: Accurately weighed 60mg of Voglibose and transfered to a 100ml volumetric flask and made up to the volume with diluent. From this 5ml was pipetted in to a 50ml volumetric flask and made up to the volume with diluent.

Solution-B: Accurately weighed 500mg of Metformin HCl was taken in to a 500ml volumetric flask and add 300ml of diluent dissolvedd to sonicate for 5min. Add 5ml of Solution-A and make up with diluent.

Filter the solution through 0.45µ membrane filter. Inject six replicated injections in to the HPLC system and calculate the RSD from six replicate injections.

I.b.SYSTEM SUITABILITY

1.Blank solution: Purified Water is used as diluent.

2.Standard preparation:

Solution-A: Accurately weighed 60mg of Voglibose and transfed to a 100ml volumetric flask and made up to the volume with diluent. From this 5ml was pipette out in to a 50ml volumetric flask and made up to the volume with same diluent.

Solution-B: Accurately weighed 500mg of Metformin HCl was taken in to a 500ml volumetric flask and add 300ml of diluent dissolvedd to sonicate for 5min. And add 5ml of Solution-A and make up with diluents.

3.Preparation of test solution: About 0.7200gm of sample was added in to a 500ml of volumetric flask and added 300ml of diluent and sonicate for 30min to obtain uniform dispersion and then volume was made up to the mark with diluent. Filtered through 0.45µm membrane filter paper.

The results are tabulated.

II. SPECIFICITY

1. Blank solution: Purified Water is used as diluents.

2. Placebo preparation:

Weigh accurately 0.720gms placebo powder and transfered to 500 ml volumetricflask then added 300ml diluent. Shake and disperse the placebo and sonicate for 30 minutes to dissolved the content and make up the volume with diluent. Filter the solution through 0.45µ membrane filter. Collect the filtrate after discarding first few ml of the filtrate.

3. Standard preparation:

Solution-A: Accurately 60mg of Voglibose was weighed and transfer in to a 100ml volumetric flask and made up to the volume with diluent. From this 5ml was pipetted out in to a 50ml volumetric flask and made up to the volume with same diluent.

Solution-B: Accurately weighed 500mg of Metformin HCl was taken in to a 500ml volumetric flask and add 300ml of diluent dissolved to sonicate for 5min. And add 5ml of Solution-A and make up with diluent.

4.Preparation of test solution: about 0.7200gm of sample was added in to a 500ml of volumetric flask and add 300ml of diluent and sonicate for 30min to obtain uniform dispersion and then volume was made up to the mark with diluent. Filtered through 0.45µ membrane filter paper.

III. PRECISION:

a) Repeatability: establish the repeatability of the analytical method by estimating the assay for six sample preparations of the same batch under normal operating conditions. Calculate the assay for all six-sample preparations and report the %RSD for the same.

1. Blank solution: Purified water used as diluents

2. Standard preparation:

Solution-A: Accurately weighed 60mg of Voglibose transfered to a 100ml volumetric flask and made up to the volume with diluent. From this 5ml was pipette out in to a 50ml volumetric flask and made up to the volume with same diluent.

Solution-B: Accurately weighed quantity of 500mg of Metformin HCl was taken in to a 500ml volumetric flask and adds 300ml of diluent dissolved to sonicate for 5min. And add 5ml of Solution-A and make up with diluent.

3.Preparation of test solution: about 0.7200gm of sample was added in to a 500ml of volumetric flask and add 300ml of diluent and sonicate for 30min to obtain uniform dispersion and then volume was made up to the mark with diluents. Filtered through 0.45µ membrane filter paper.

b)Intermediate precision (ruggedness): intermediate precision study was carried out by repeating the complete experiment with different analysts, on different days in same laboratory as per the following preparation.

1.Blank solution: Purified water used as diluent

2. Standard preparation:

Solution-A: Accurately weighed 60mg of Voglibose transferred to a 100ml volumetric flask and made up to the volume with diluent. From this 5ml was pipette out in to a 50ml volumetric flask and made up to the volume with same diluent.

Solution-B: Accurately weighed quantities 500mg of Metformin HCl was taken in to a 500ml volumetric flask and add 300ml of diluent dissolved to sonicate for 5min. And add 5ml of Solution-A and make up with diluent.

3.Preparation of test solution: about 0.7200gm of sample was added in to a 500ml of volumetric flask and add 300ml of diluent and sonicate for 30min to obtain uniform dispersion and then volume was made up to the mark with diluent. Filtered through 0.45µ membrane filter paper.

IV. LINEARITY:

Preparation of standard stock solution:

Accurately weighed 60mg of Voglibose working standard transferred to 100ml volumetric flask and dissolved with diluent and make up the volume (solution A). Accurate weigh 5000mg of Metformin Hcl working standard and transfered to 500ml volumetric flask and dissolved it in 300ml of diluent. Take 5ml of above solution A and transfered into a 500ml of Metformin Hcl volumetric flask and make up the volume with diluent.

Preparation of linearity solution (50%): Pipette out 5ml from stock solution in to a clean 100ml volumetric flask and make up to volume with diluent.

Preparation of linearity solution (75%): Pipette out 7.5ml from stock solution in to a clean 100ml volumetric flask and make up to volume with diluent.

Preparation of linearity solution (100%): Pipette out 10ml from stock solution in to a clean 100ml volumetric flask and make up to volume with diluent.

Preparation of linearity solution (125): Pipette out 12.5ml from stock solution in to a clean 100ml volumetric flask and make up to volume with diluent.

Preparation of linearity solution (150%): Pipette out 15ml from stock solution in to a clean 100ml volumetric flask and make up to volume with diluent.

Run the solutions as described above.

V. ACCURACY/ RECOVERY:

Preparation of standard stock solution-A:

Accurately weighed 60mg of Voglibose working standard and transfered in to a 100ml volumetric flask and dissolved with diluent and make up the volume with diluent (solution A).

Preparation of linearity solution (50%): Accurately weighed 50mg of Metformin HCl in to a 100ml volumetric flask containing about 145mg of placebo and 5ml of Solution-A and added 70ml of diluent and sonicate about 30min to dissolved the content and make up the volume with diluent. Filter the solution through 0.45µ membrane filter. Repeat this procedure for another two sample preparations.

Preparation of linearity solution (75%): Accurately weighed 75mg of Metformin HCl in to a 100ml volumetric flask containing about 145mg of placebo and 7.5ml of Solution-A and added 70ml of diluent and sonicate about 30min to dissolved the content and make up the volume with diluent. Filter the

solution through 0.45µ membrane filter. Repeat this procedure for another two sample preparations.

Preparation of linearity solution (100%): Accurately weighed 100mg of Metformin HCl in to a 100ml volumetric flask containing about 145mg of placebo and 10ml of Solution-A and add 70ml of diluent and sonicate about 30min to dissolved the content and make up the volume with diluent. Filter the solution through 0.45µ membrane filter. Repeat this procedure for another two sample preparations.

Preparation of linearity solution (125%):Accurately weighed about 125mg of Metformin HCl in to a 100ml volumetric flask containing about 145mg of placebo and 12.5ml of Solution-A and add 70ml of diluent and sonicate about 30min to dissolved the content and make up the volume with diluent. Filter the solution through 0.45µ membrane filter. Repeat this procedure for another two sample preparations.

Preparation of linearity solution (150%): Accurately weighed quantity about 150mg of Metformin HCl in to a 100ml volumetric flask containing about 145mg of placebo and 15ml of Solution-A and add 70ml of diluent and sonicate about 30min to dissolved the content and make up the volume with diluent. Filter the solution through 0.45µ membrane filter. Repeat this procedure for another two sample preparations.

VI. STABILITY OF ANALYTICAL SOLUTIONS:

To establish the stability of analytical solutions by injecting the standard and sample solutions at periodic intervals up to 24hours.

1. Blank solution: Purified water used as diluents.

2. Standard preparation:

Solution-A: Accurately weighed 60mg of Voglibose transfered in to a 100ml volumetric flask and made up to the volume with diluent. From this 5ml was pipette out in to a 50ml volumetric flask and made up to the volume with same diluent.

Solution-B: Accurately weighed quantity 500mg of Metformin HCl was taken in to a 500ml volumetric flask and add 300ml of diluent dissolved to sonicate for 5min. And add 5ml of Solution-A and make up with diluent.

3. Preparation of test solution: about 0.7200gm of sample was added in to a 500ml of volumetric flask and add 300ml of diluent and sonicate for 30min to obtain uniform dispersion and then volume was made up to the mark with diluent. Filtered through 0.45µ membrane filter paper.

VII. ROBUSTNESS:

To determine the robustness of the analytical method. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and provides an indication of its suitability during normal usage. Perform the study by evaluating the system suitability parameter and estimating the assay under deliberately modified chromatographic conditions.

Deliberately modified chromatographic conditions: Deliberately modify the actual chromatographic conditions specified under the method like flow rate, mobile phase composition, column temperature on lower and higher side of the actual value. Evaluate system suitability and determine the assay of Metformin HCl and Voglibose under these

1. Blank solution: Purified water used as diluent

2. Standard preparation:

Solution-A: Accurately weighed 60mg of Voglibose transfered in to a 100ml volumetric flask and made up to the volume with diluent. From this 5ml was

pipette out in to a 50ml volumetric flask and made up to the volume with same diluent.

Solution-B: Accurately weighed quantitiy 500mg of Metformin HCl was taken in to a 500ml volumetric flask and add 300ml of diluent dissolved to sonicate for 5min. And add 5ml of Solution-A and make up with diluent.

3. Preparation of test solution: about 0.7200gm of sample was added in to a 500ml of volumetric flask and add 300ml of diluent and sonicate for 30min to obtain uniform dispersion and then volume was made up to the mark with diluent. Filtered through 0.45µ membrane filter paper. Robustness parameters

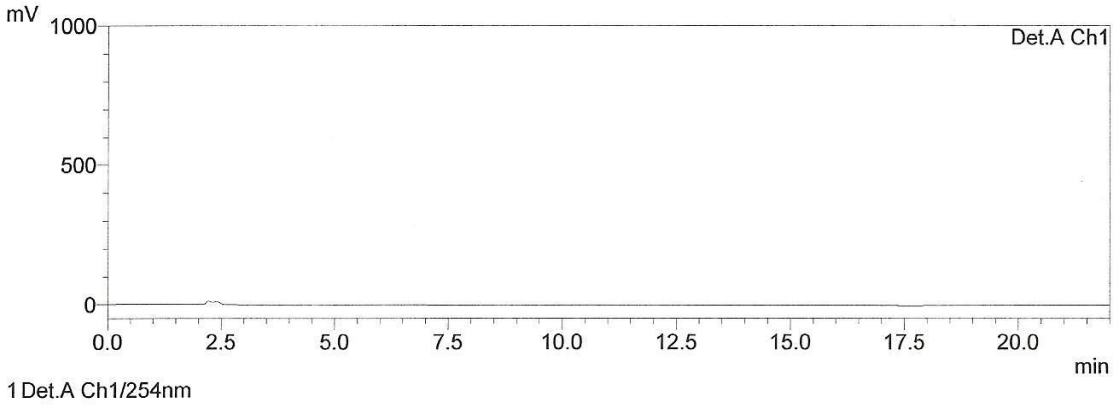
S.No	Chromatographic parameter	Low	High
1	Flow Rate(1.0ml/min)	0.8ml	1.2ml
2	Column Temperature(25^{o}C)	23^{o}C	27^{o}C
3	Mobile phase composition(Buffer: Acetonitrile 380:620)	400:600	360:640
4	Buffer pH(6.5)	6.3	6.7

Separately inject 20µl of blank, standard and sample preparations in to the chromatograph set under deliberately modified chromatographic conditions are record the chromatograms. Measure the peak responses.

CHROMATOGRAMS

II. SPECIFICITY:

Fig- Blank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Det.A Ch1
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7.5
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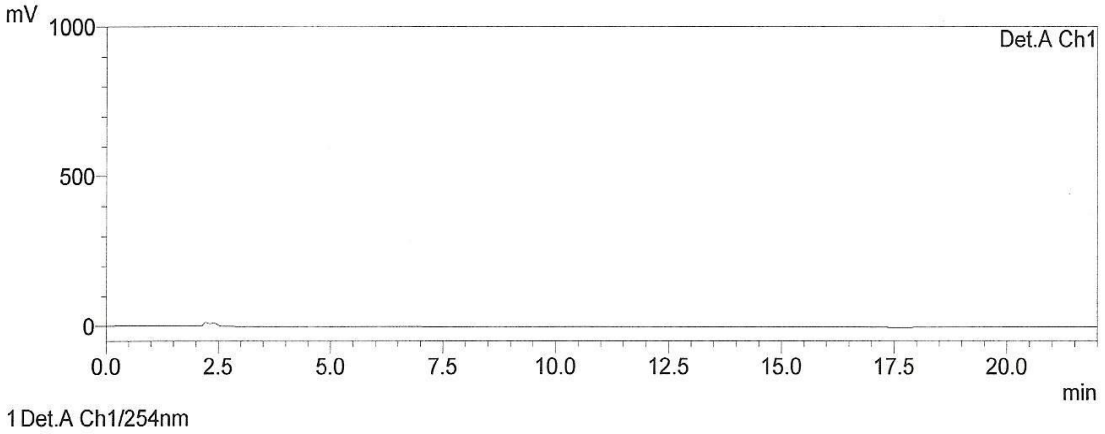
20.0
min
1 Det.A Ch1/254nm

Fig- Placebo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min
1 Det.A Ch1/254nm

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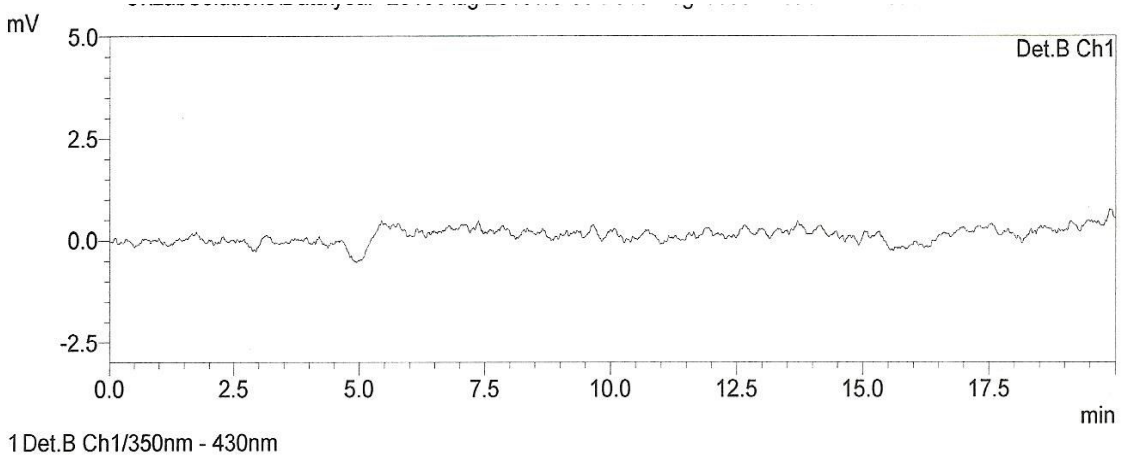


Fig- Metformin HCl

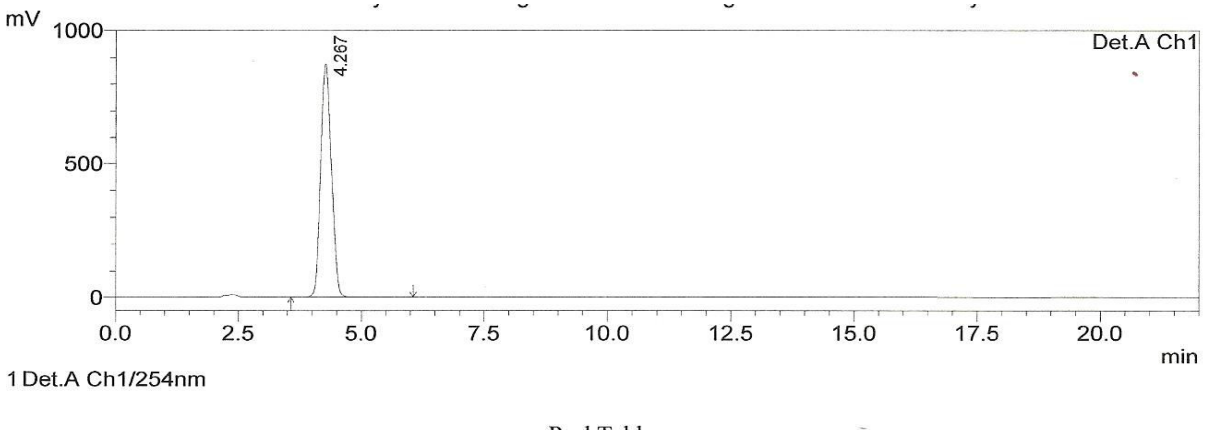
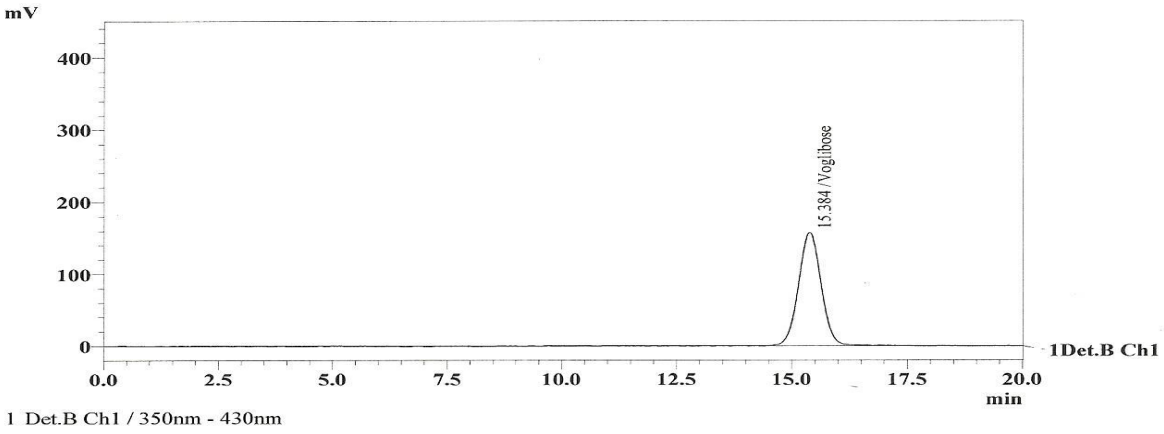


Fig- Voglibose



III.PRECISION:

a) Repeatability:

Fig- Metformin HCl

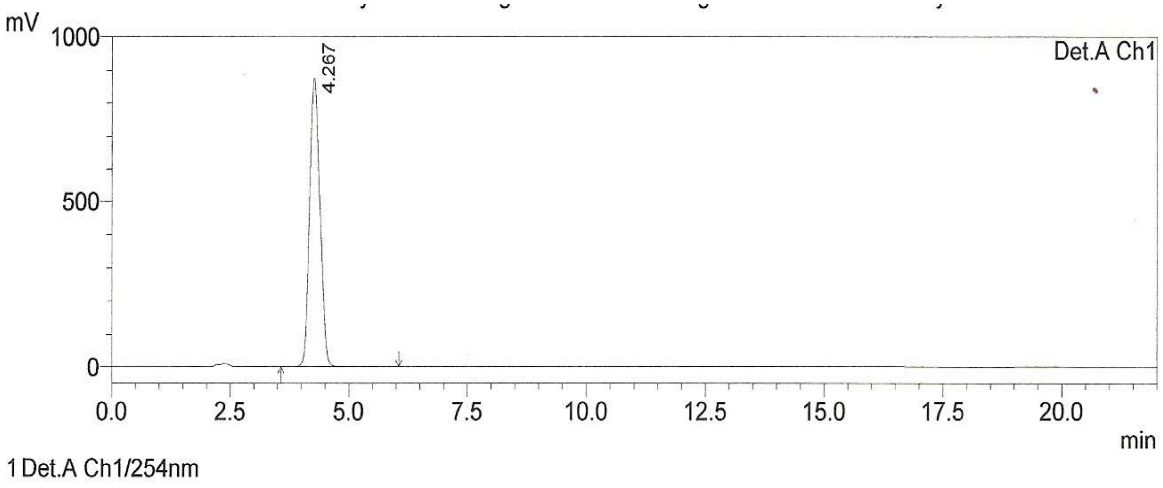
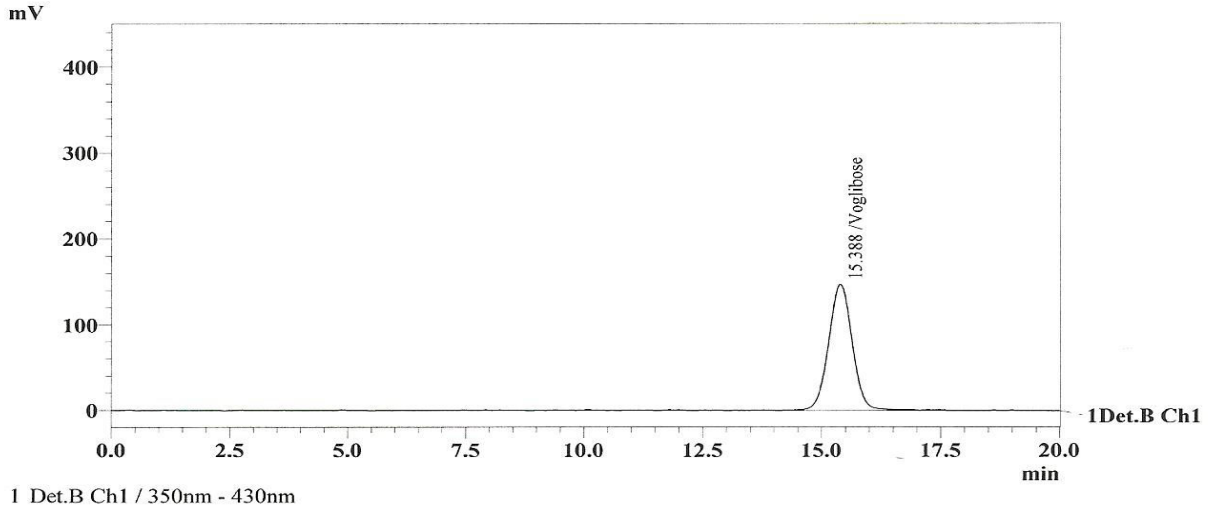


Fig- Voglibose



b) Intermediate Precision (Day to Day):

Fig- Metformin HCl:

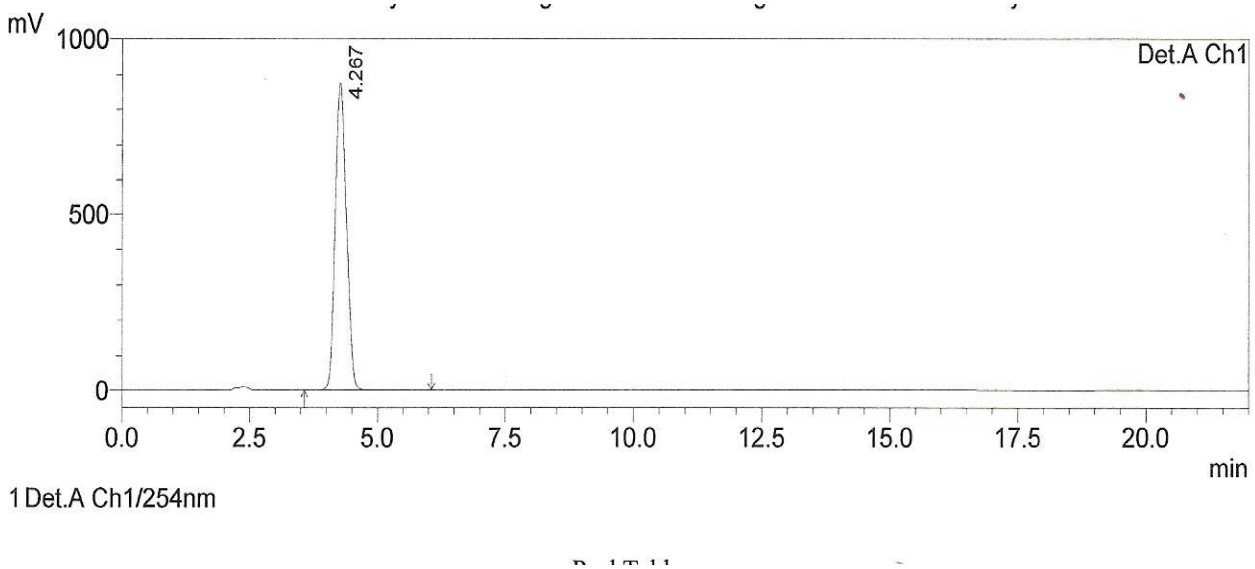
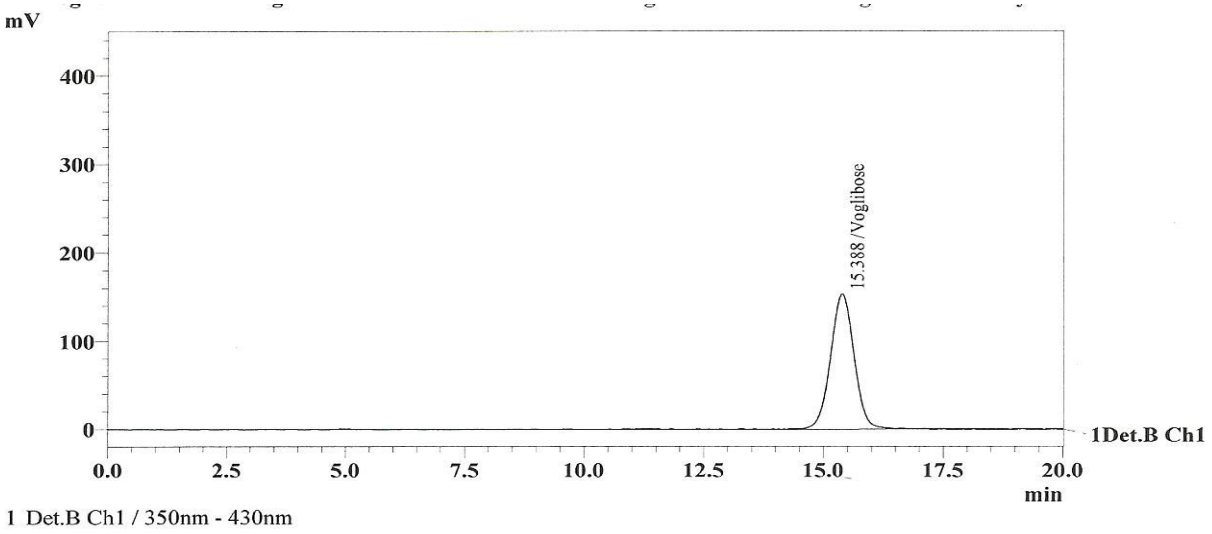


Fig- Voglibose



LINEARITY:

Metformin HCl:

Fig- Metformin HCl 50%

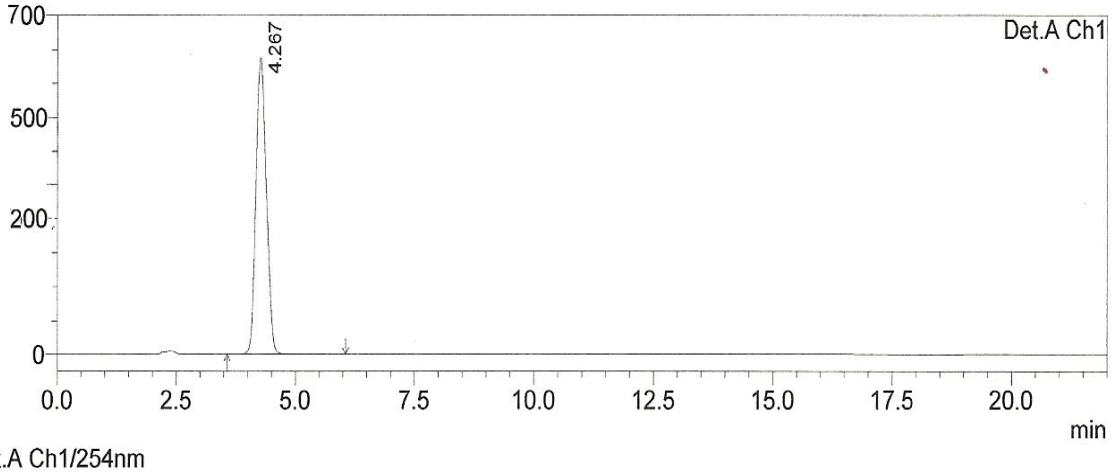


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Fig- Metformin HCl 75%

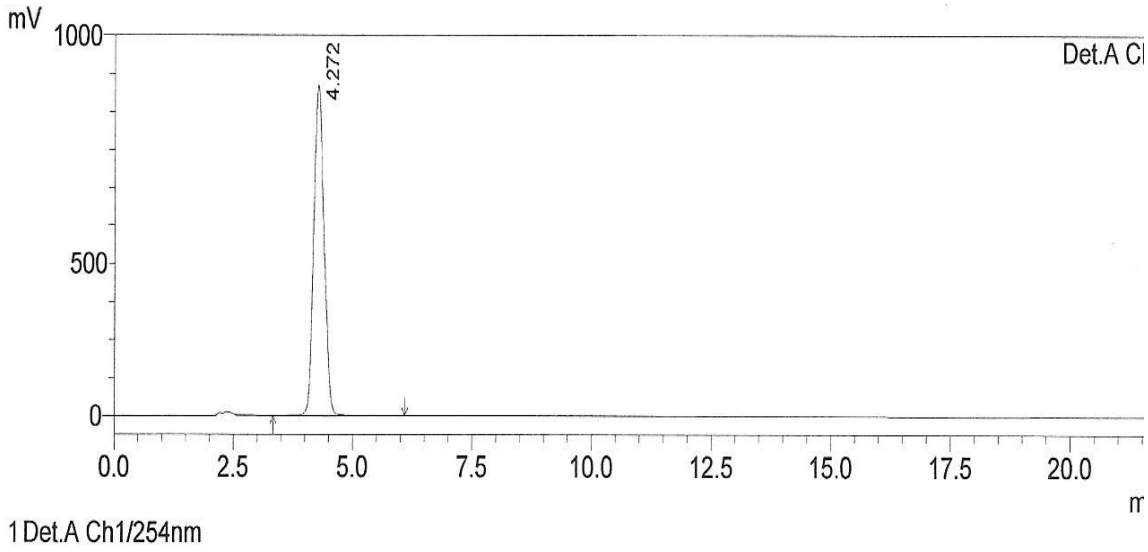

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Fig- Metformin HCl 100%

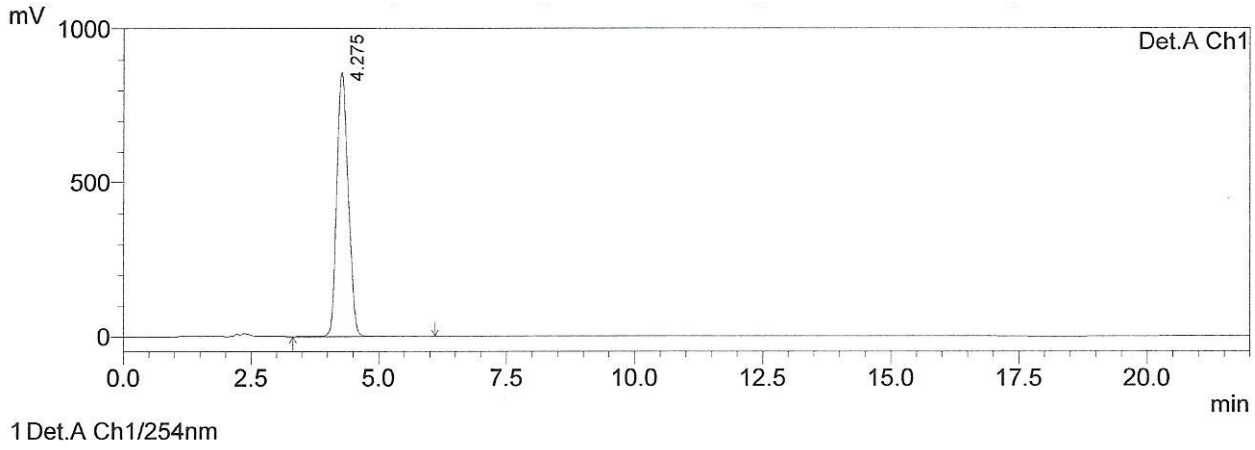


Fig- Metformin HCl 125%

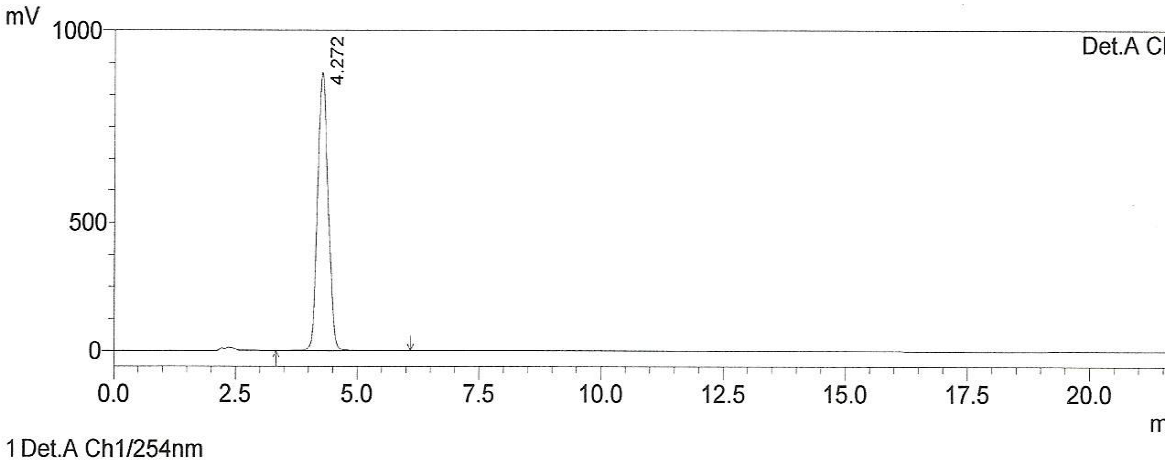
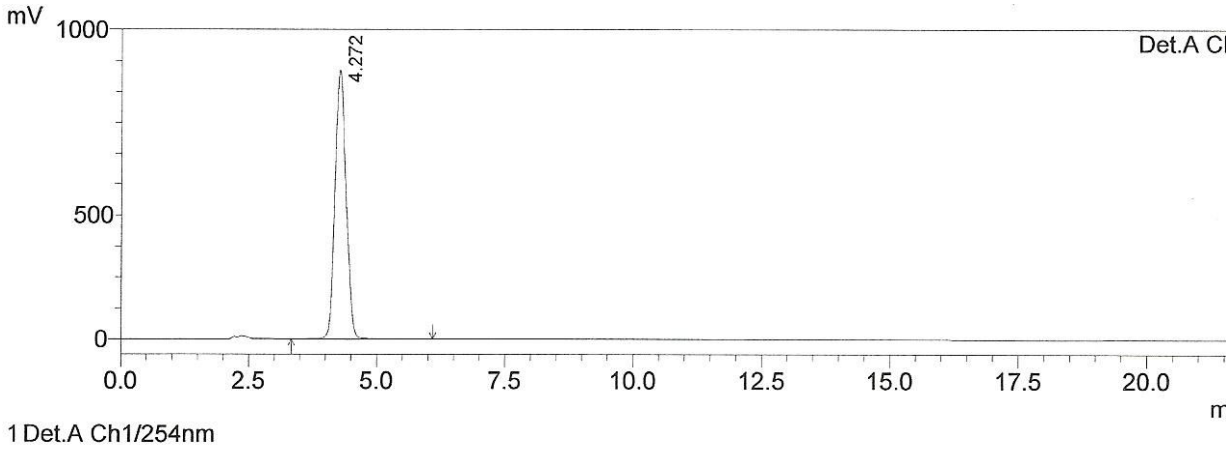
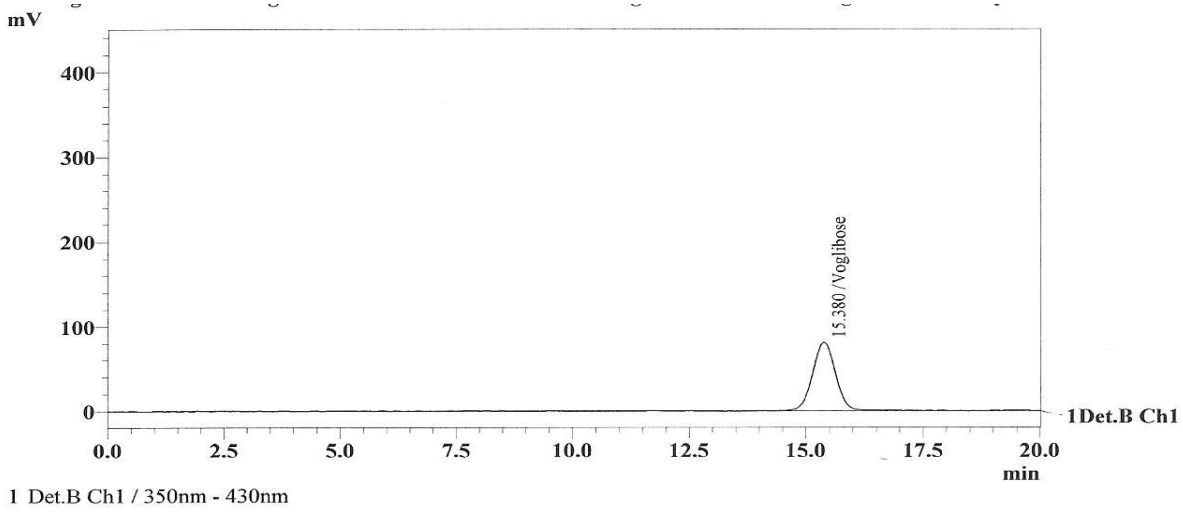


Fig- Metformin HCl 150%



Voglibose:

Fig- Voglibose 50%



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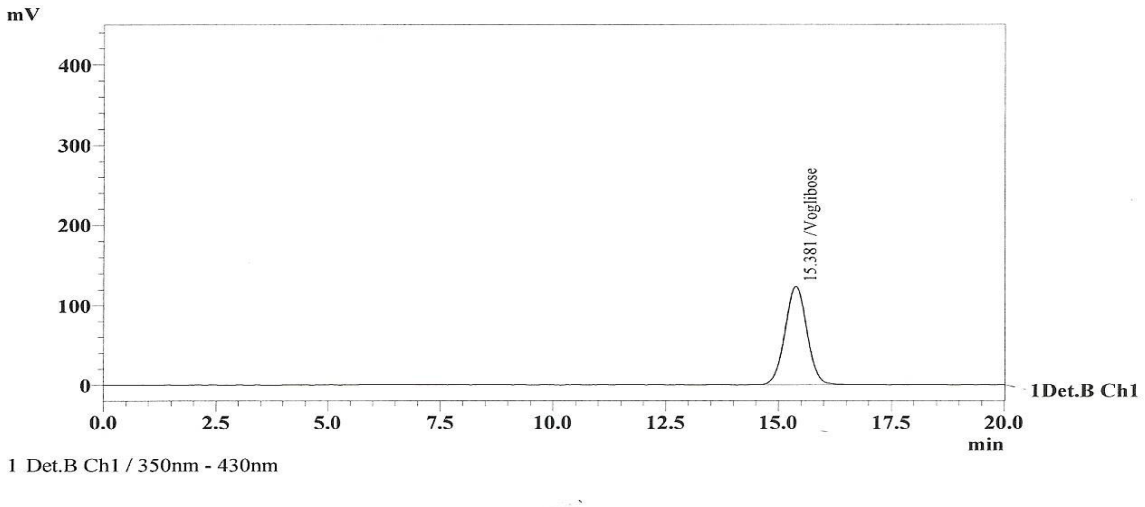

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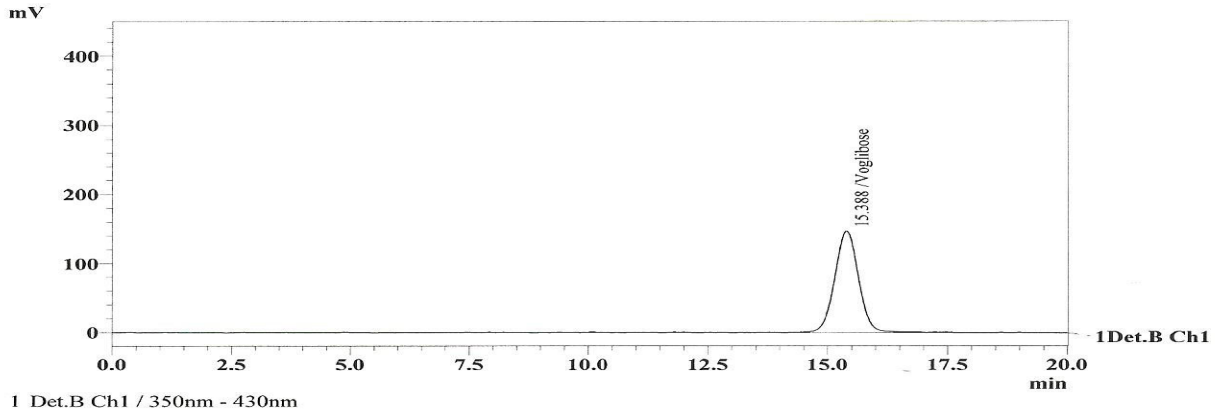

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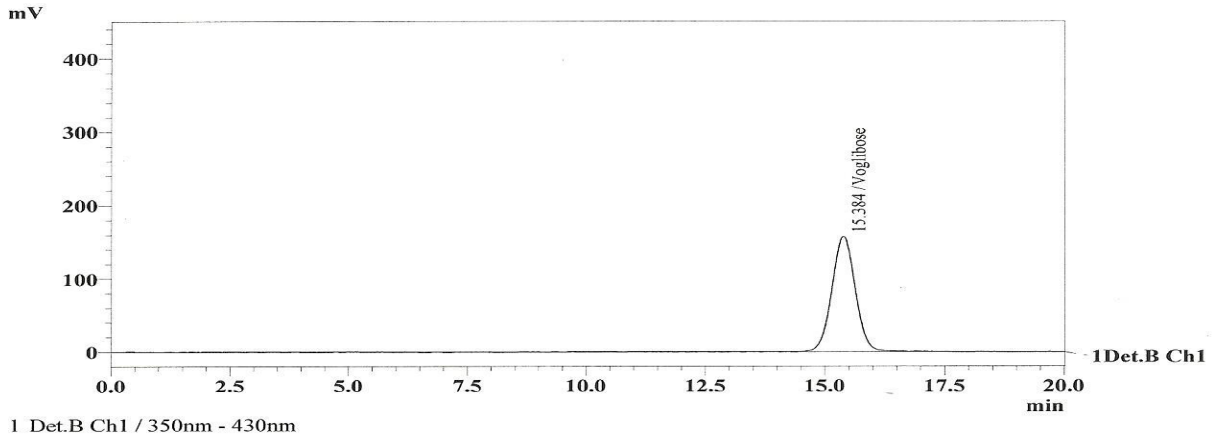

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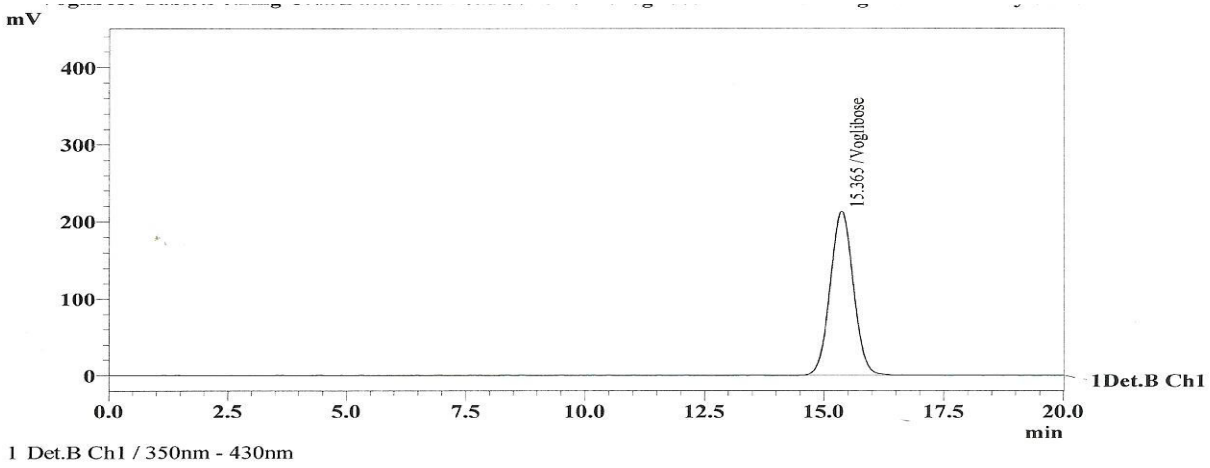

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ACCURACY/RECOVERY:

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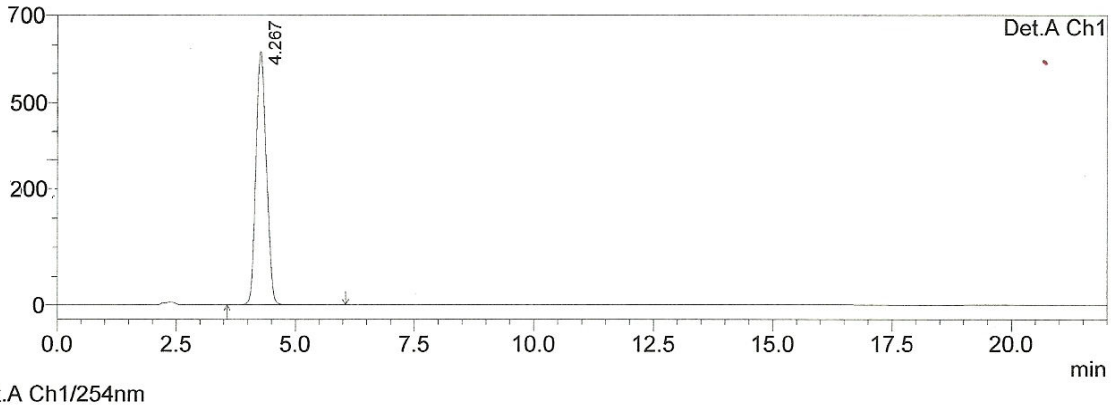

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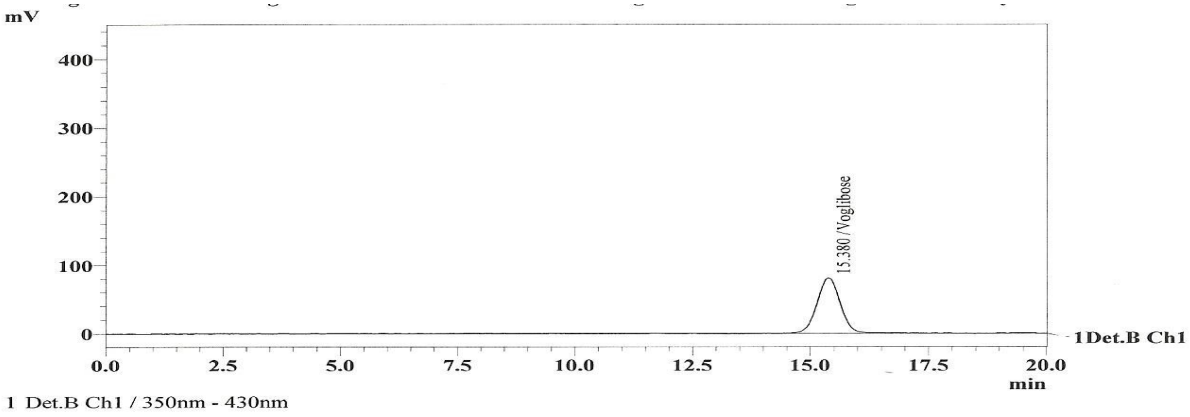

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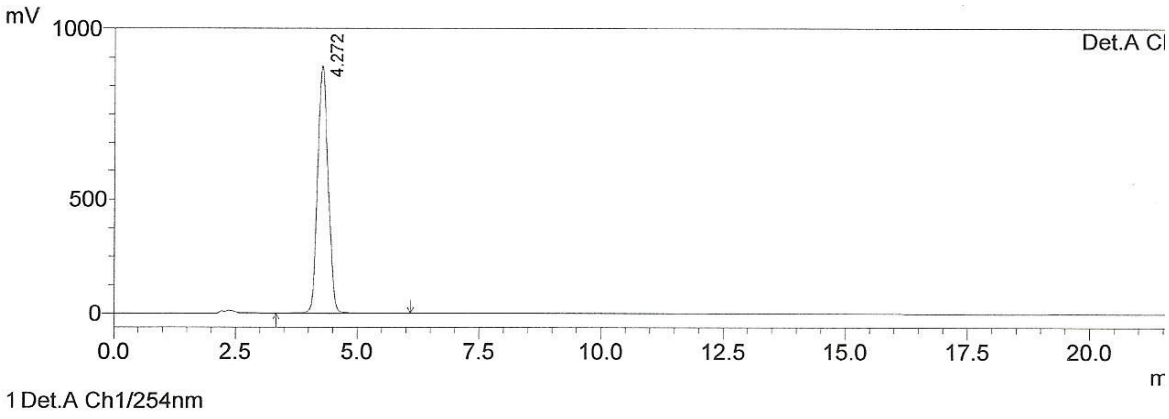

Fig-: Voglibose 75%

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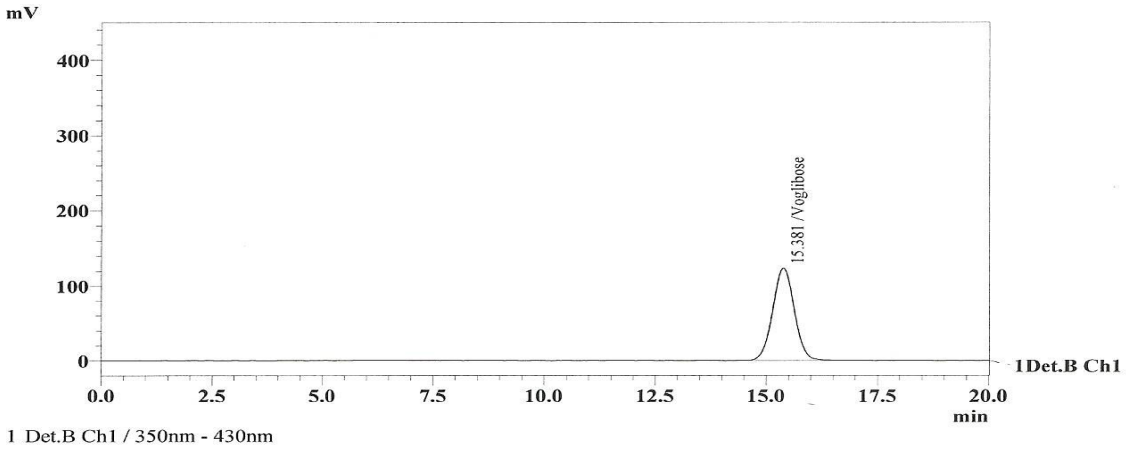

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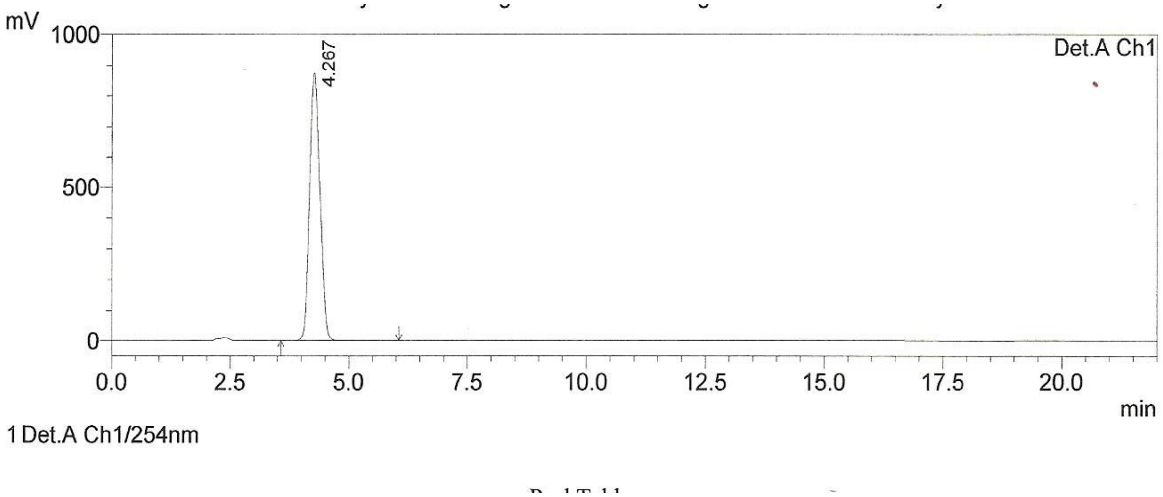

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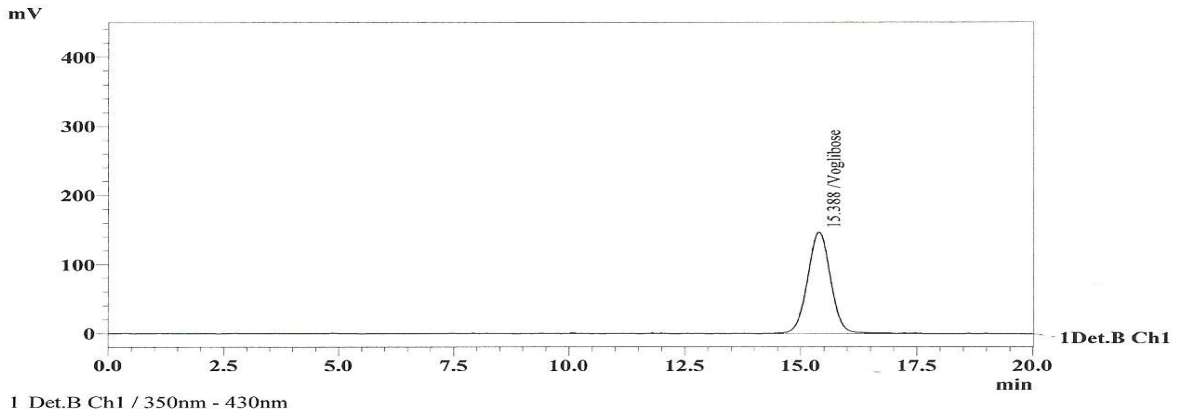

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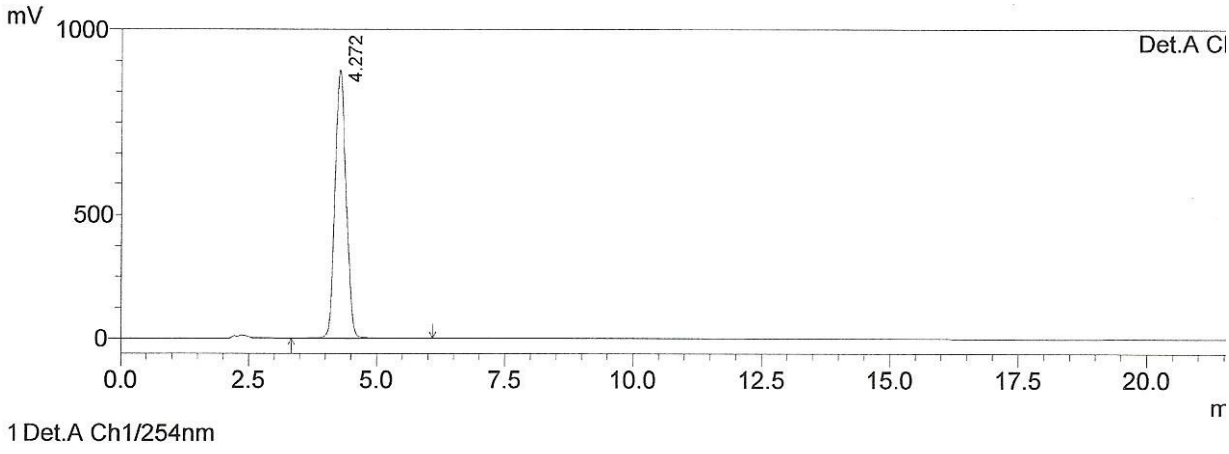

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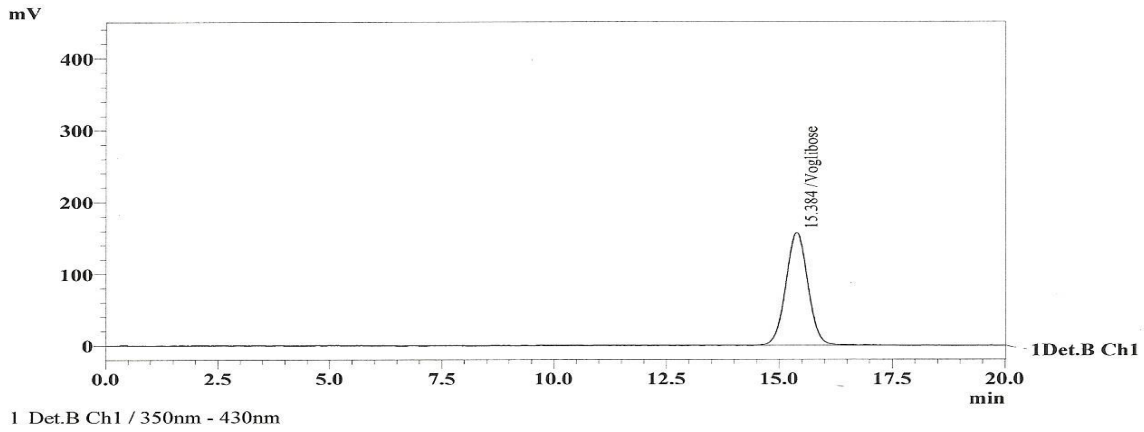

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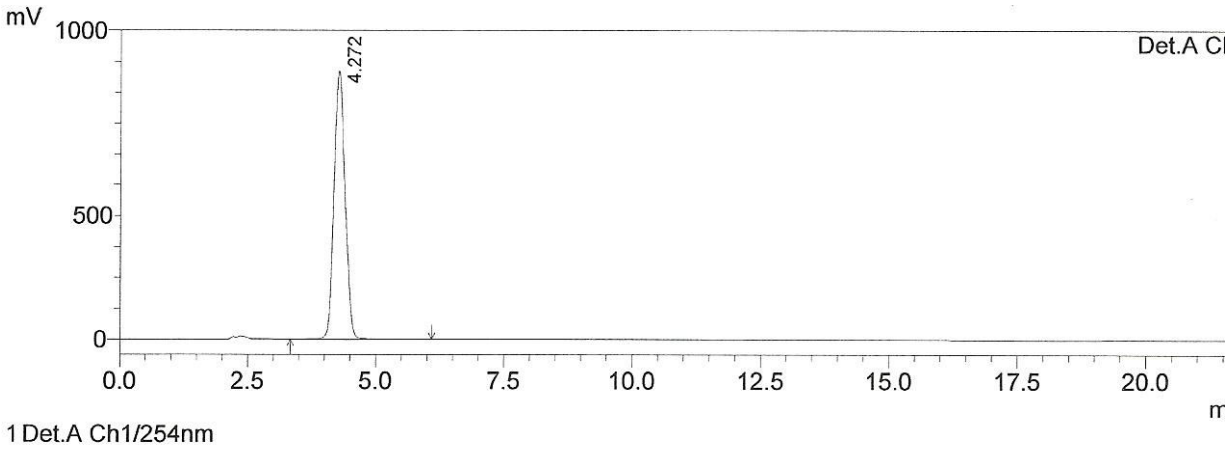

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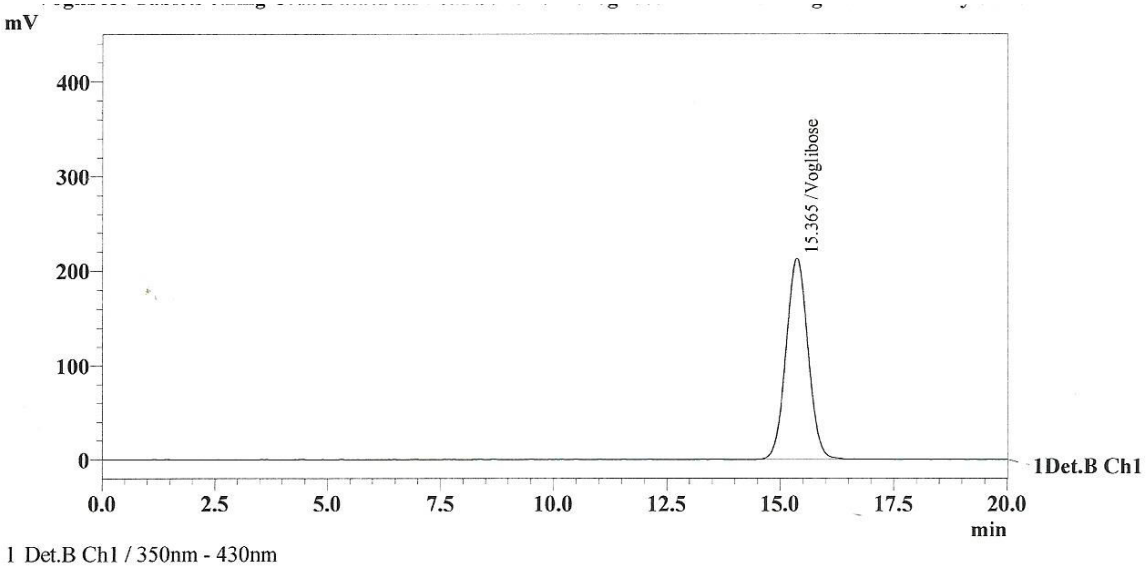

STABILITY OF ANALYTICAL SOLUTIONS:

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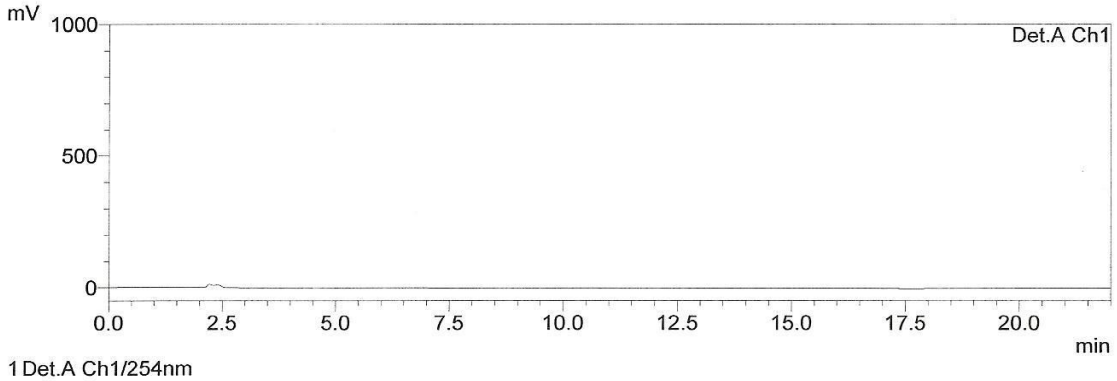
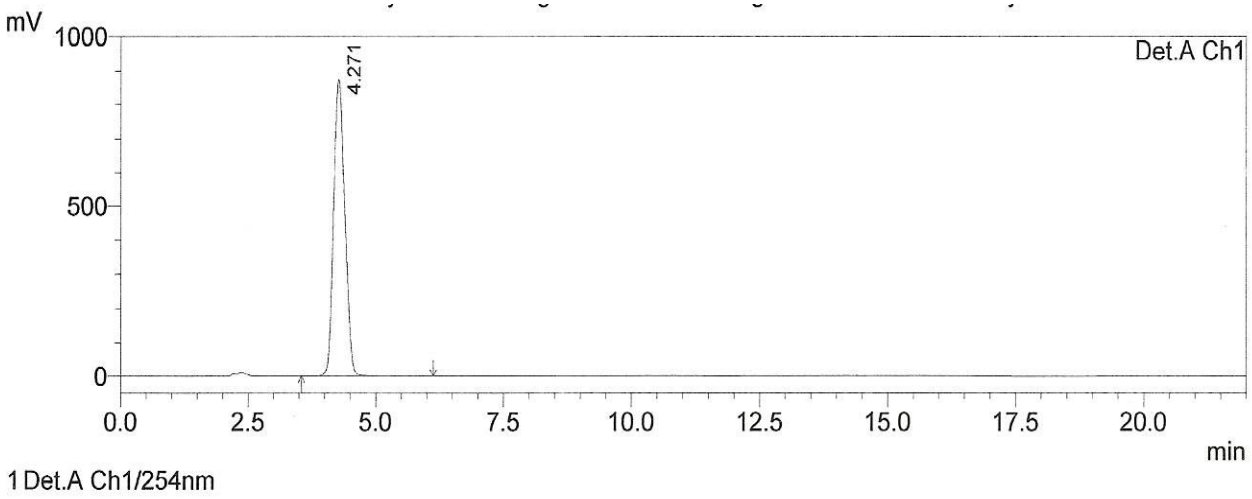


Fig-: 0th hour- Metformin HCl



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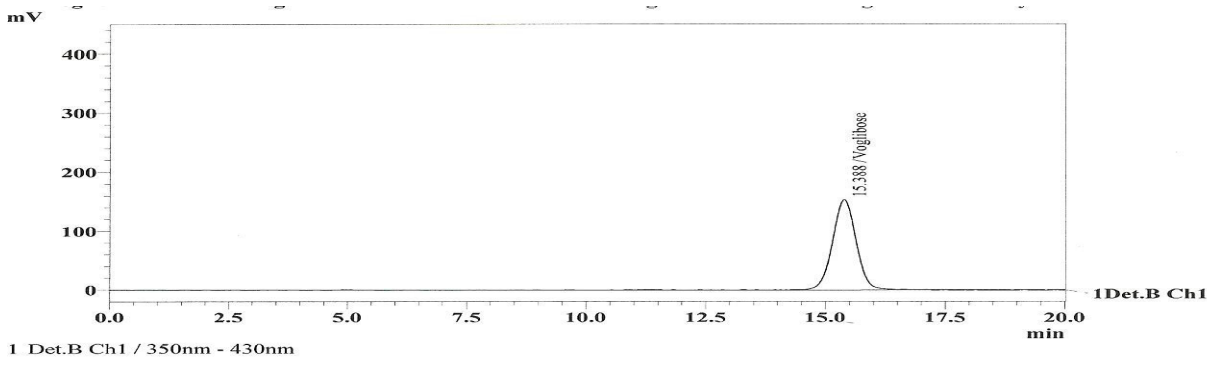
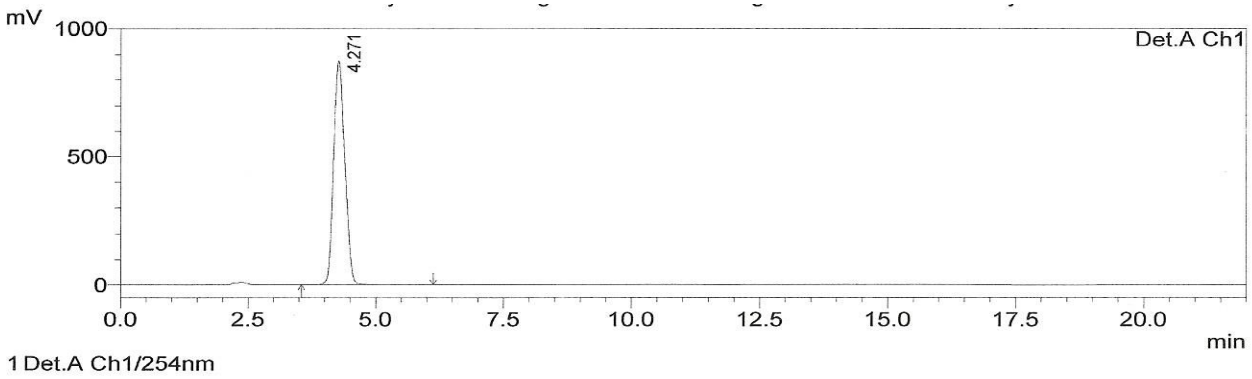


Fig- 2nd hour- Metformin HCl



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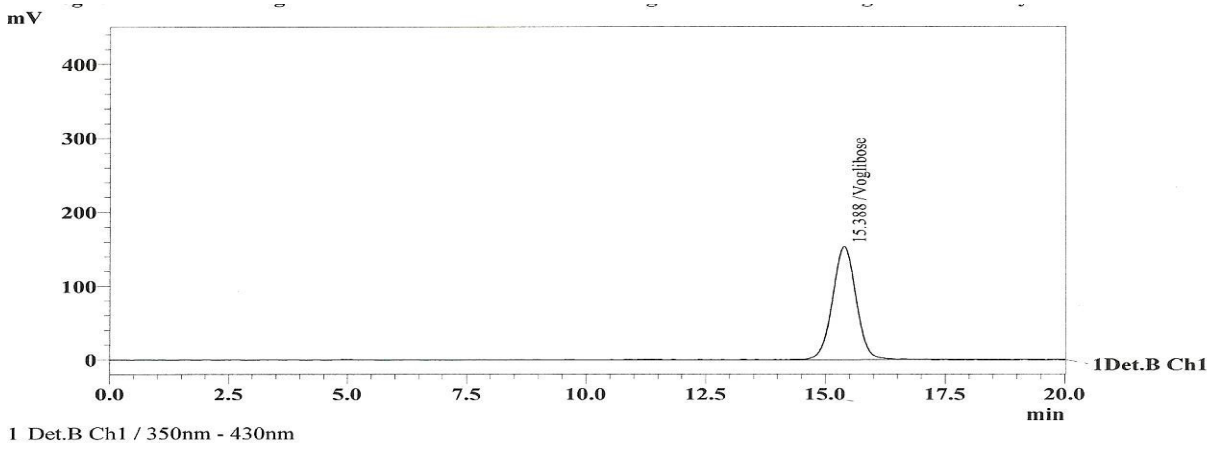


Fig- 4th hour-Metformin HCl

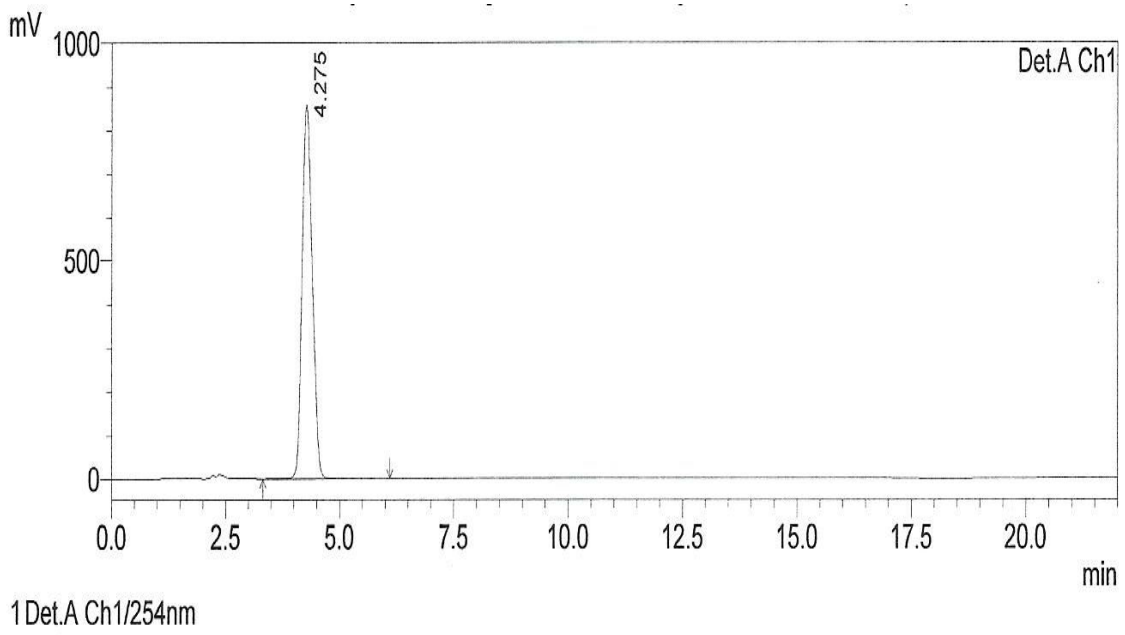


Fig-: Voglibose

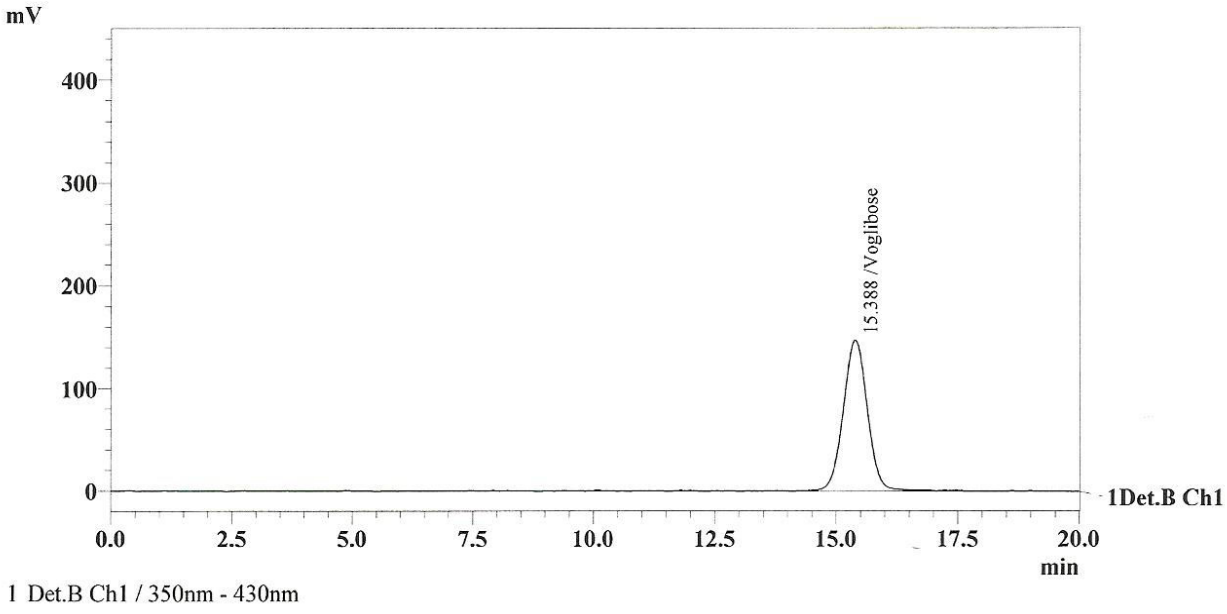


Fig-: 8th hour- Metformin HCl

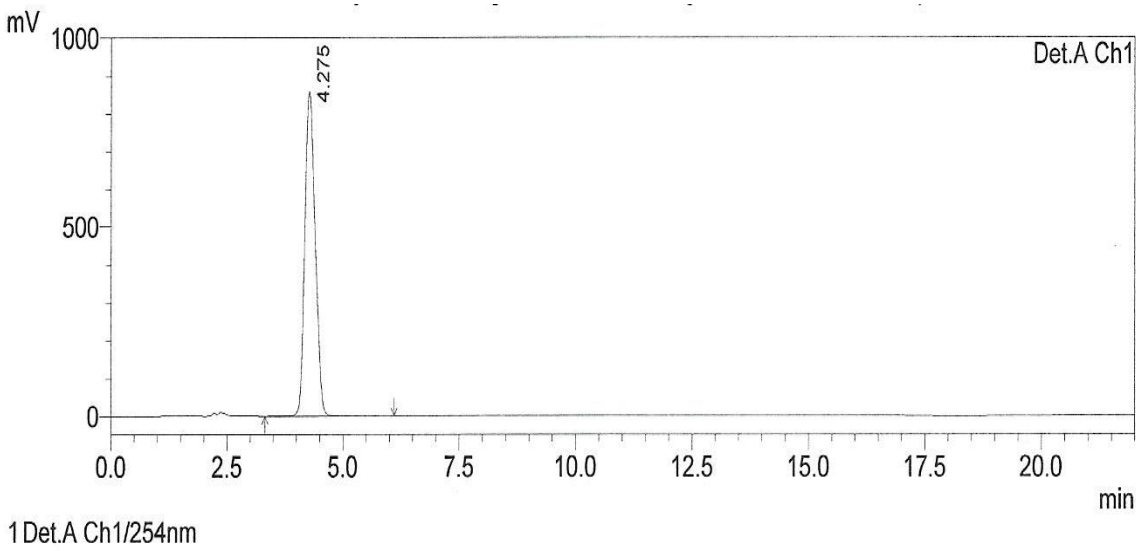


Fig- : Voglibose

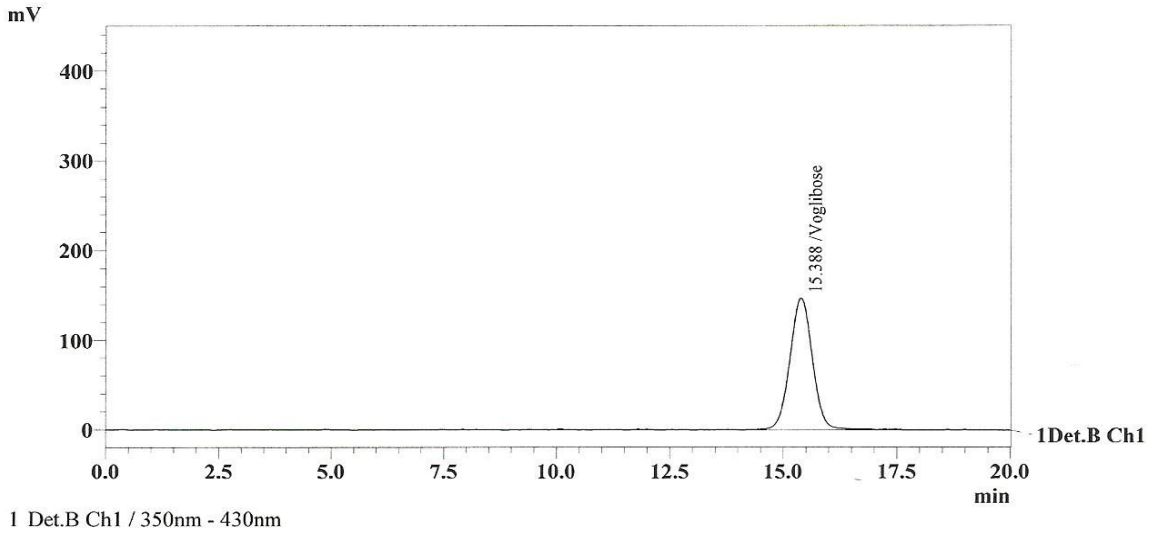


Fig-: 12th hour- Metformin HCl

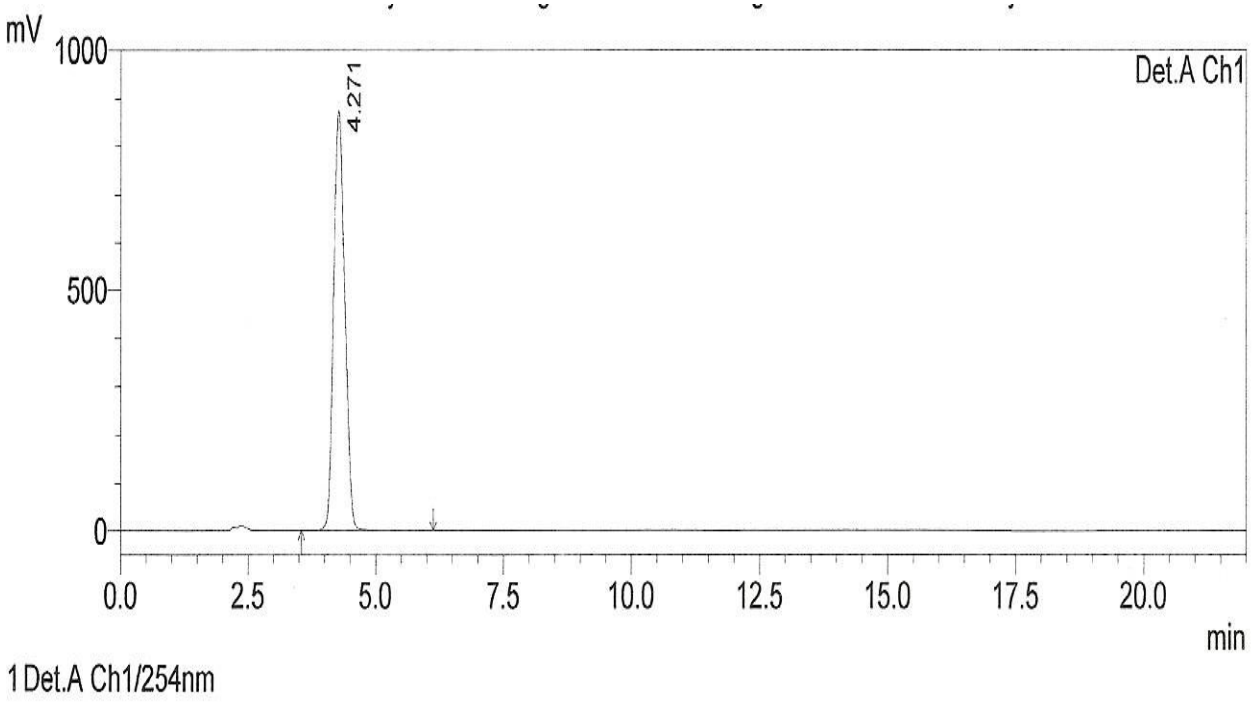


Fig-: Voglibose

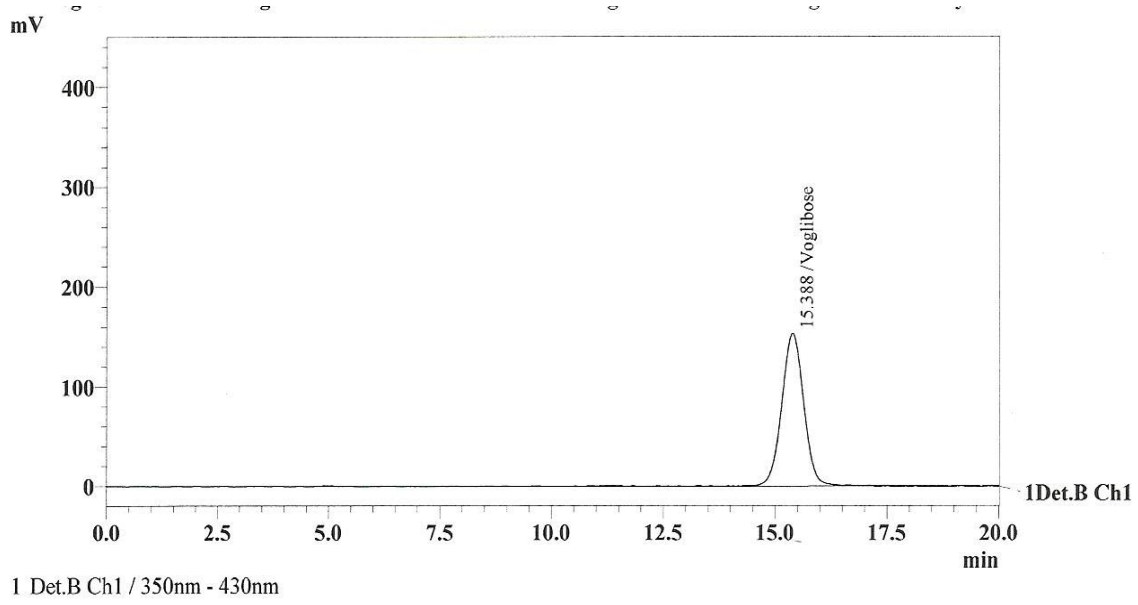


Fig-: 18th hour- Metformin HCl

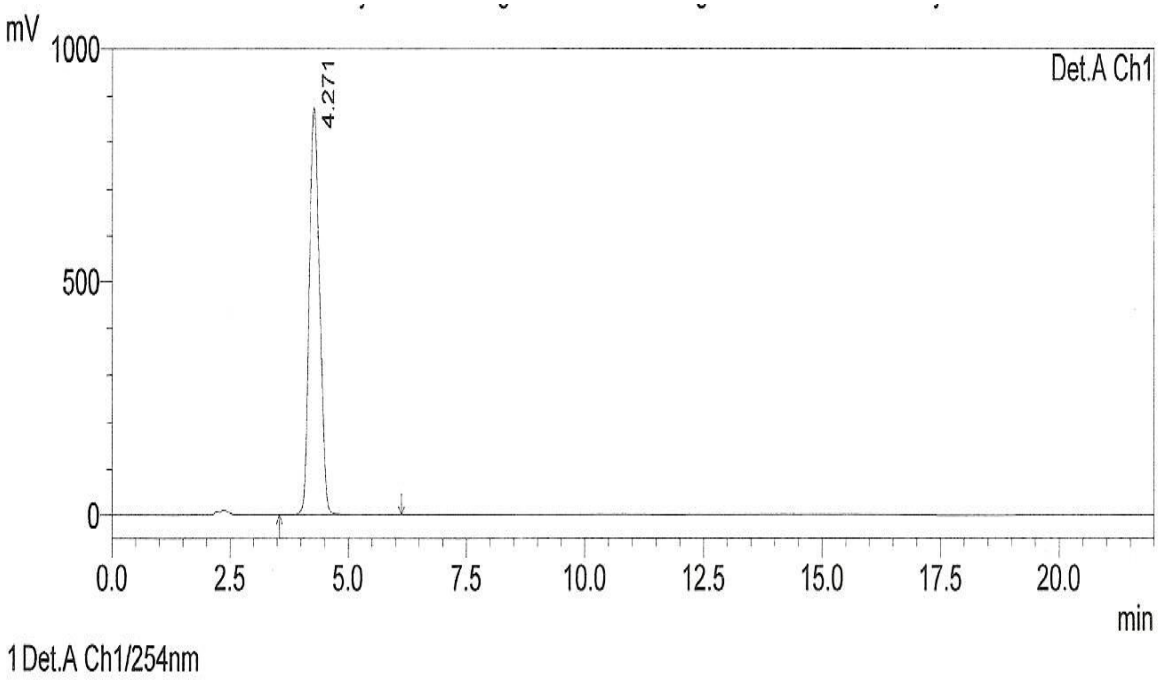


Fig-: Voglibose

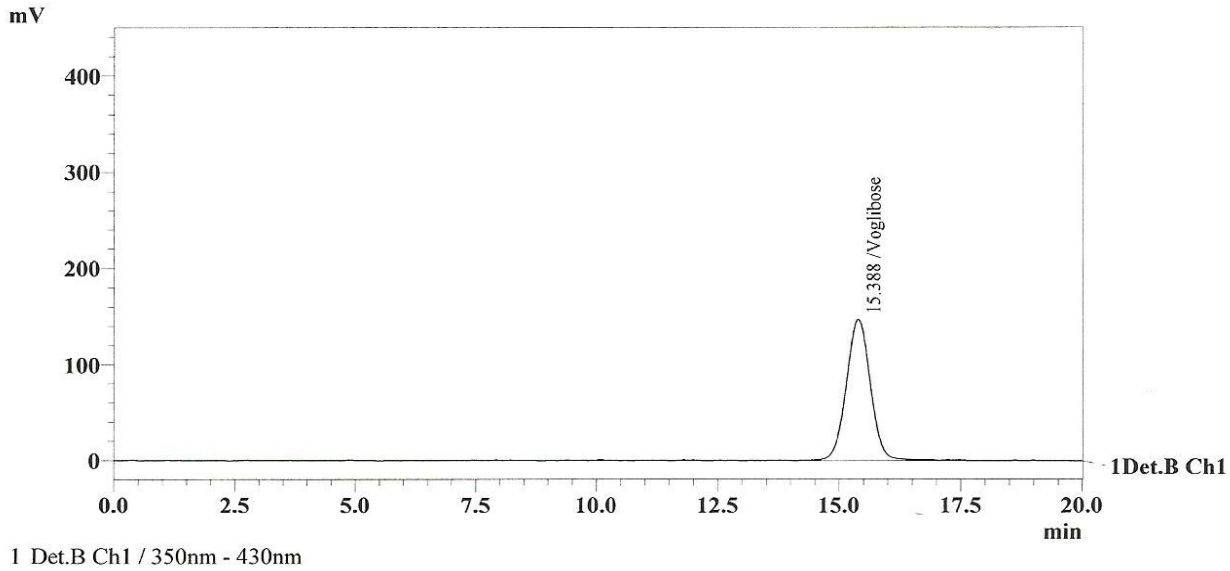


Fig- 24th hour-Metformin HCl

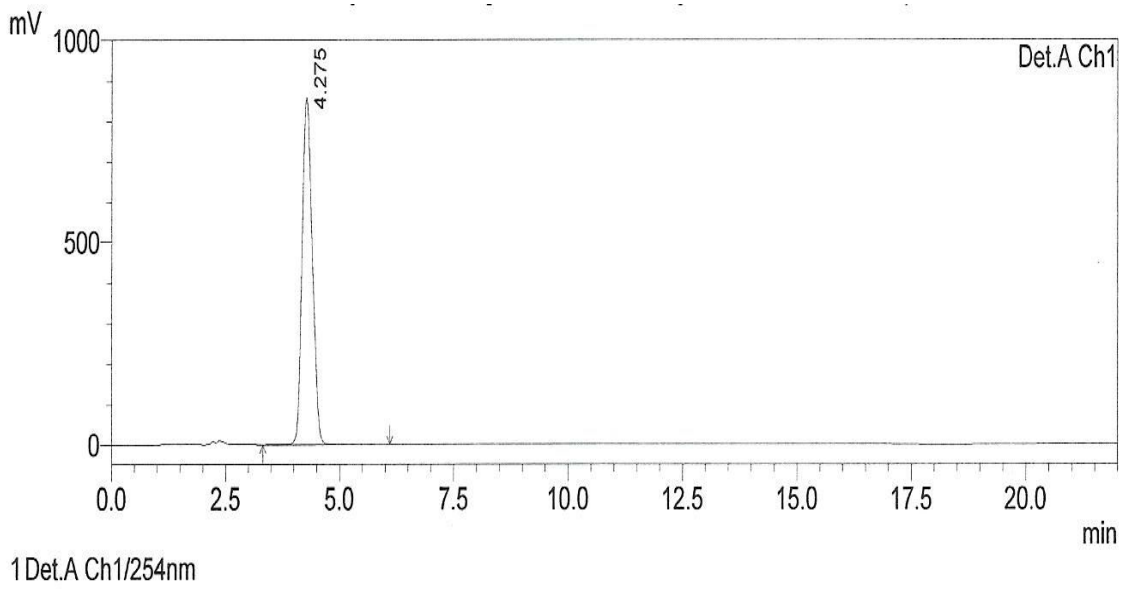
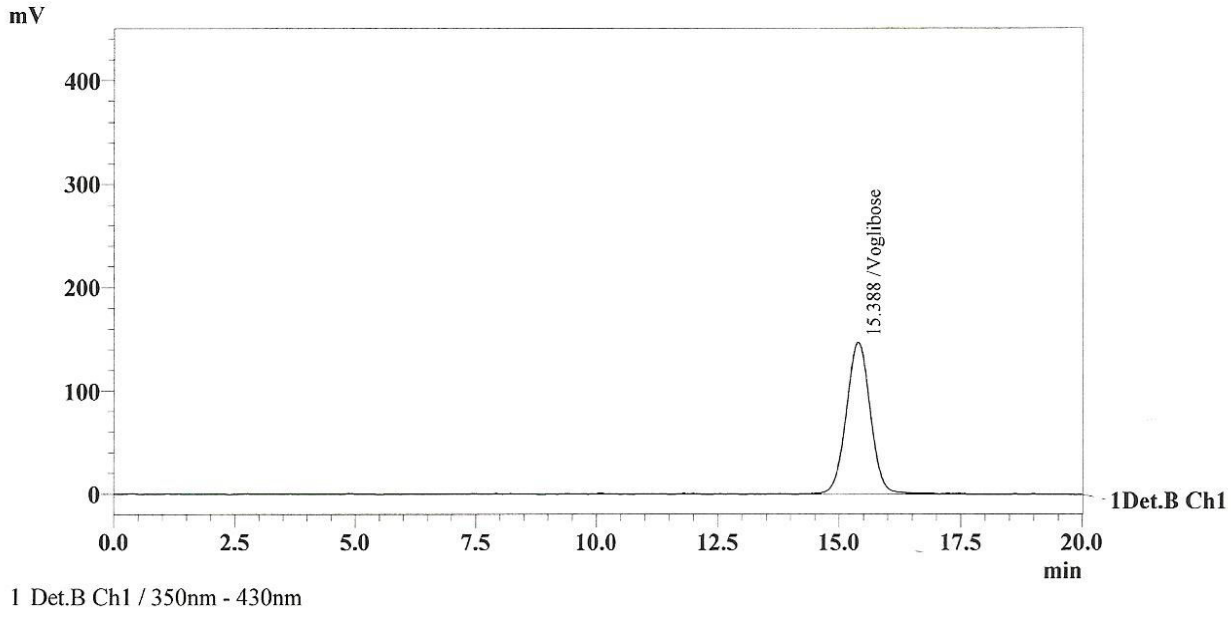


Fig-: Voglibose



ROBUSTNESS:

Robustness of High flow (1.2ml):

Fig-: Metformin HCl

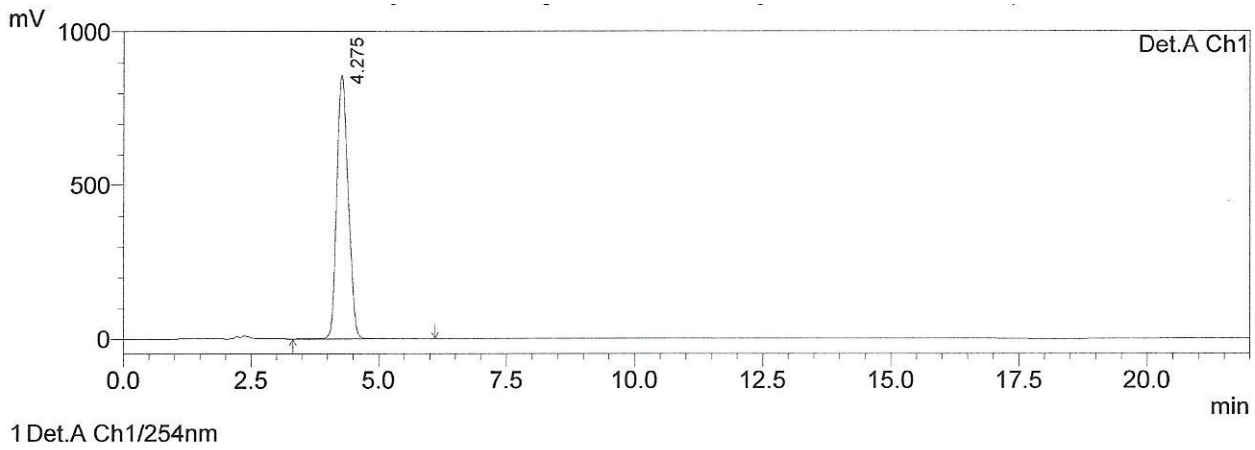
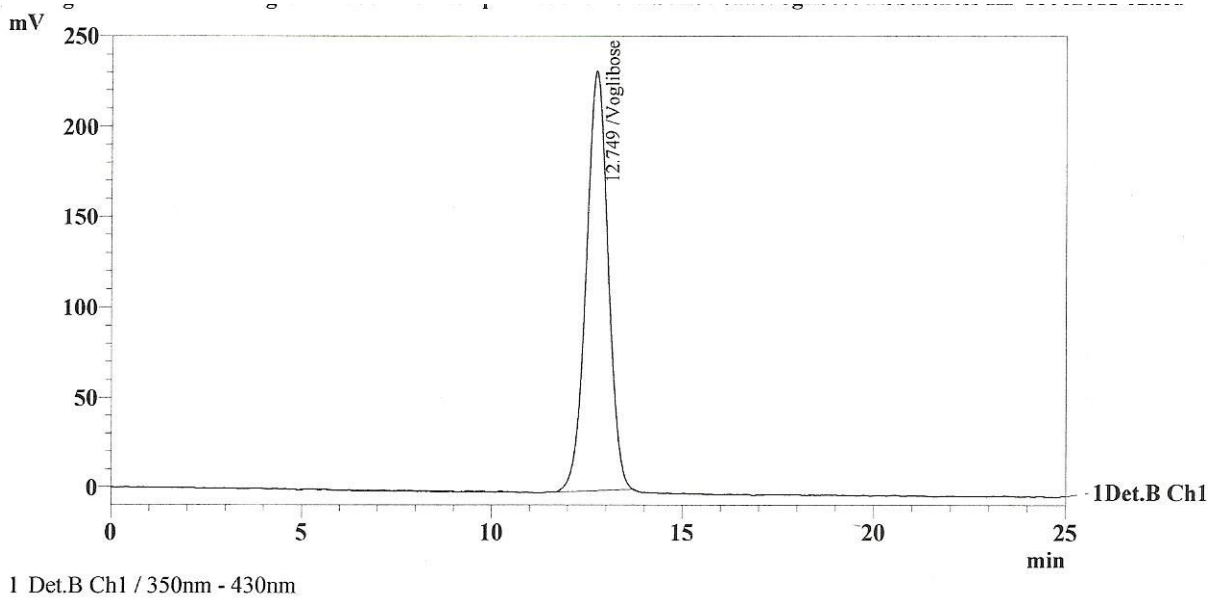


Fig-: Voglibose



Robustness of Low flow (0.8ml):

Fig-: Metformin HCl

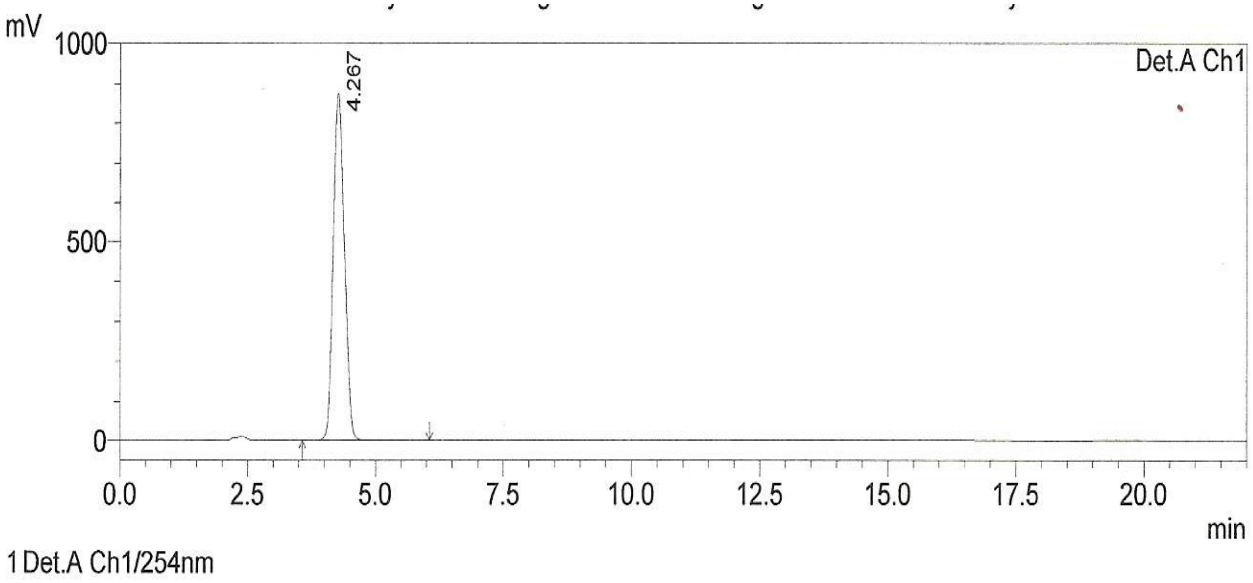
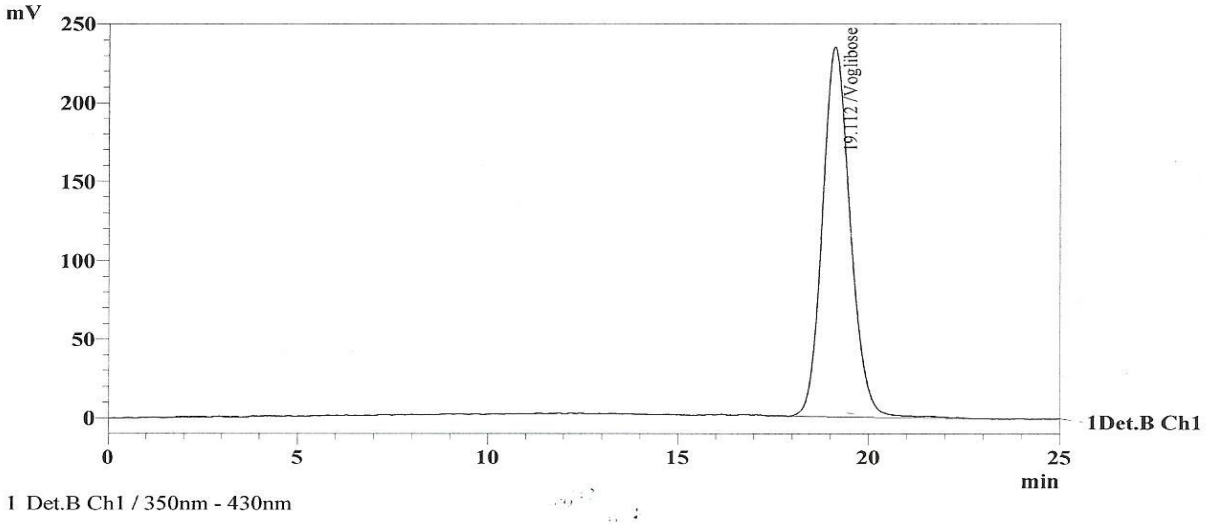


Fig-: Voglibose



Robustness of High Temp (27 C):

Fig-: Metformin HCl

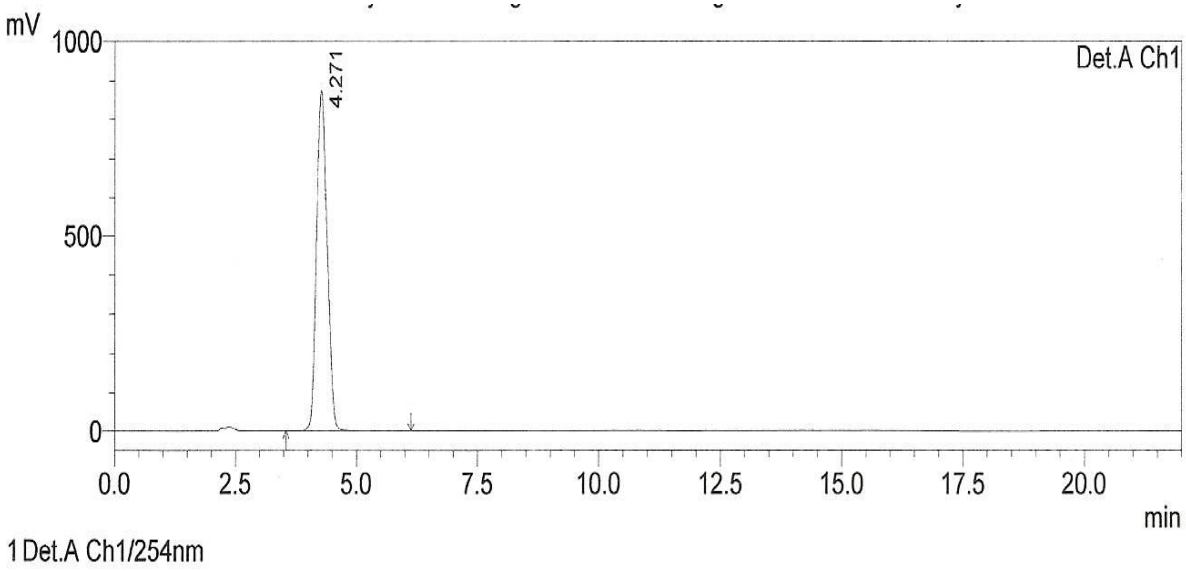


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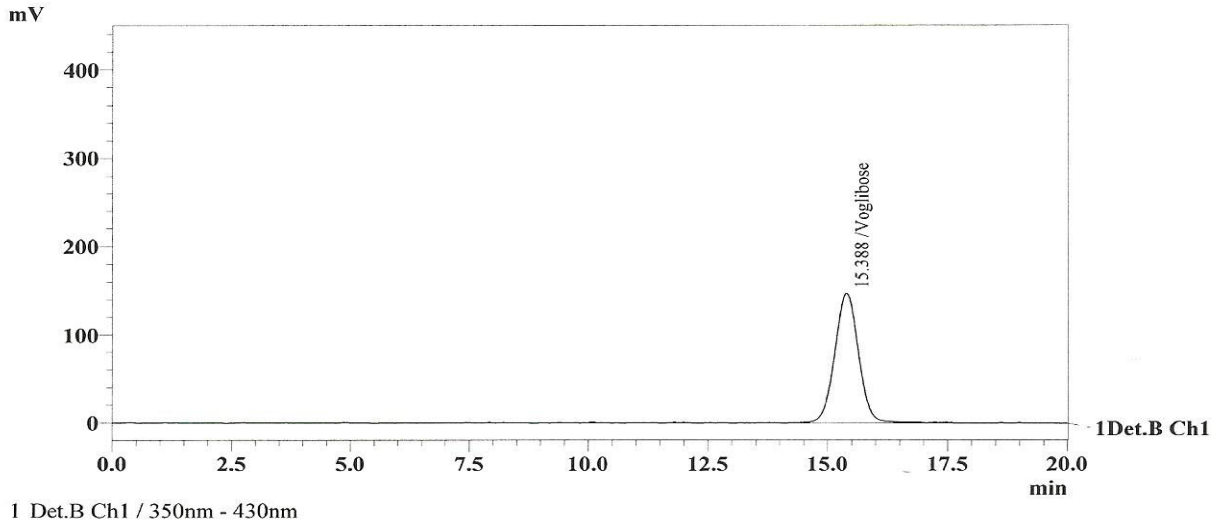
Fig-: Voglibose



Robustness of Low Temp (23 C):

Fig-: Metformin HCl

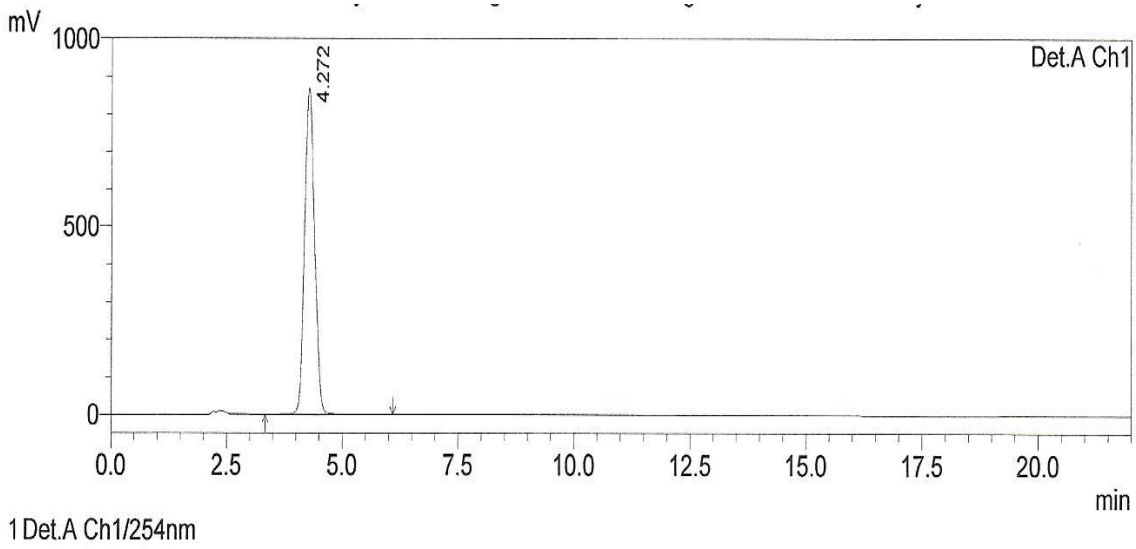


Fig-: Voglibose

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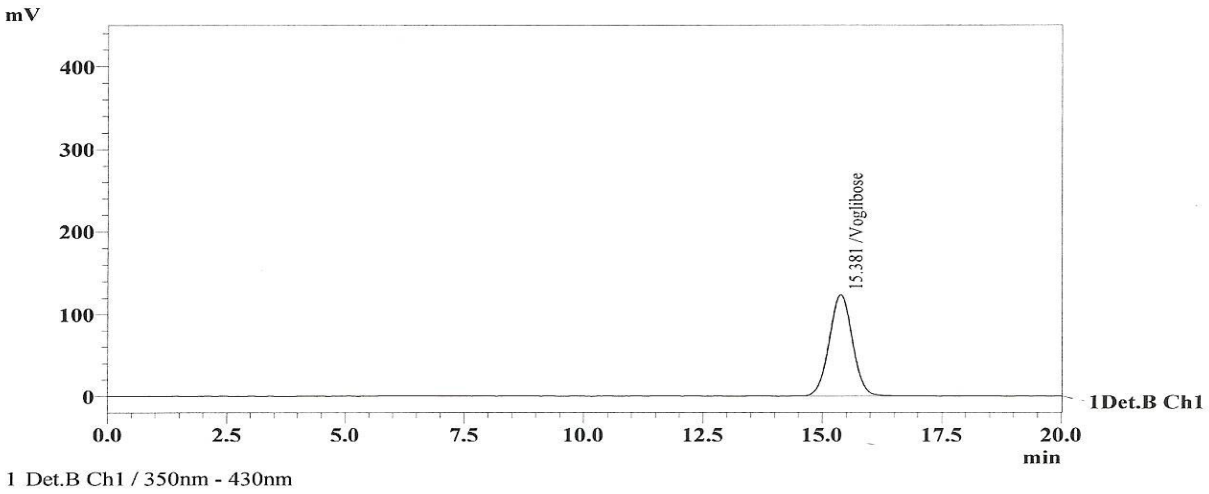


Robustness of Mobile phase Changes (Buffer:ACN 400:600):

Fig-: Metformin HCl

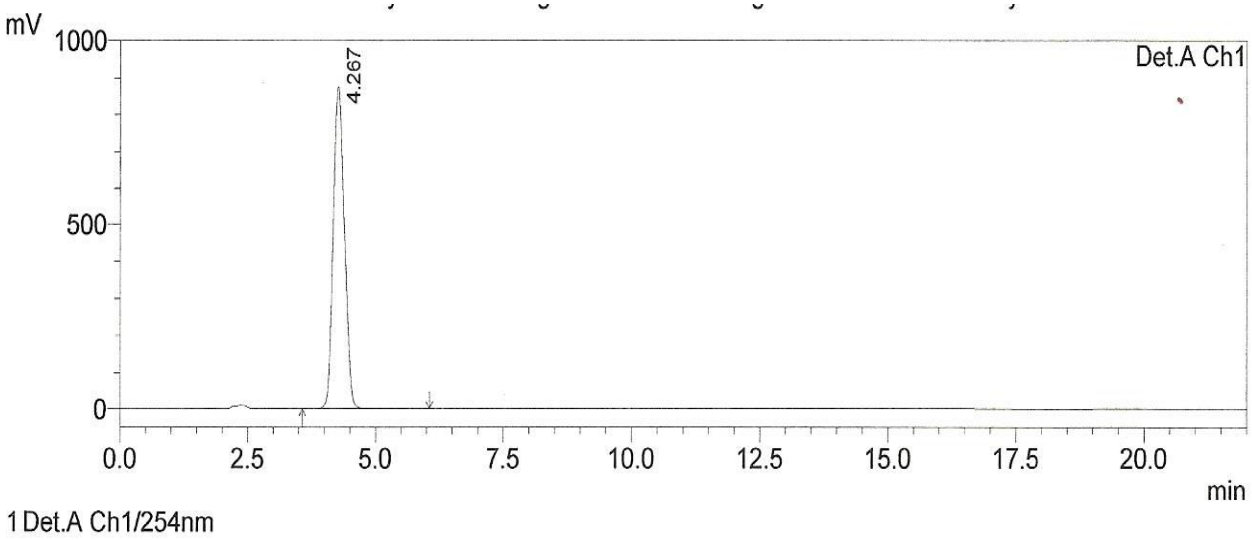
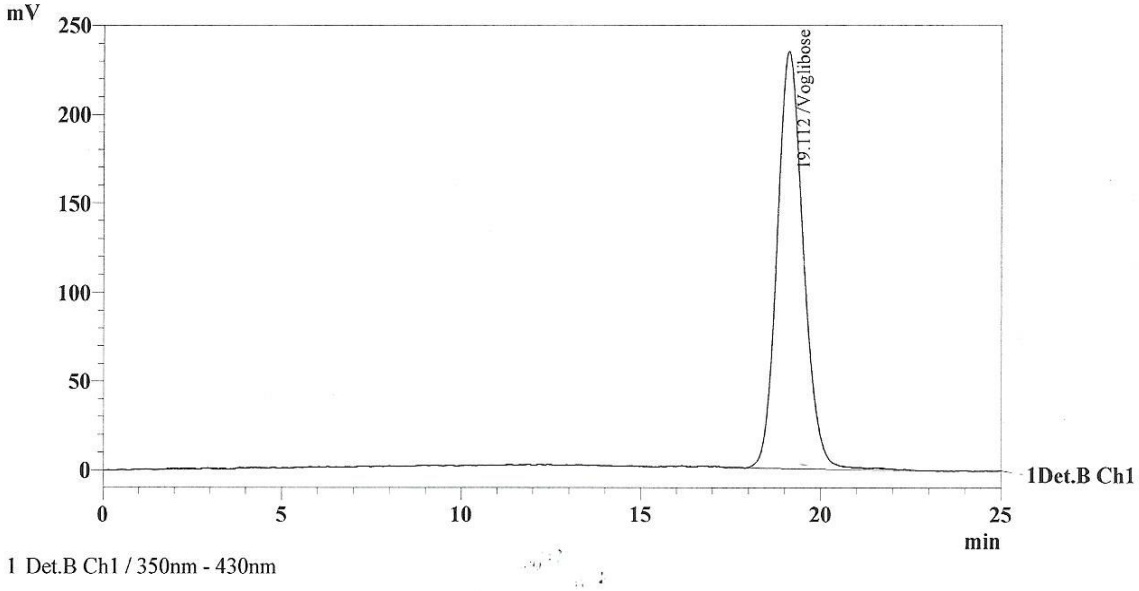


Fig-: Voglibose



Robustness of Mobile phase Changes (Buffer:ACN 360:640):

Fig-: Metformin HCl

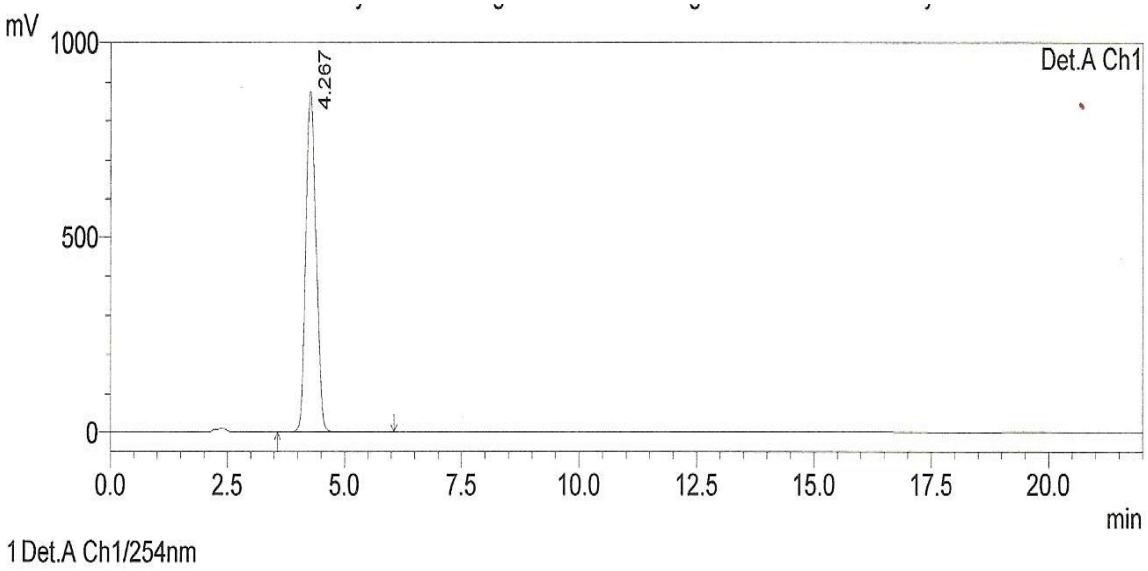
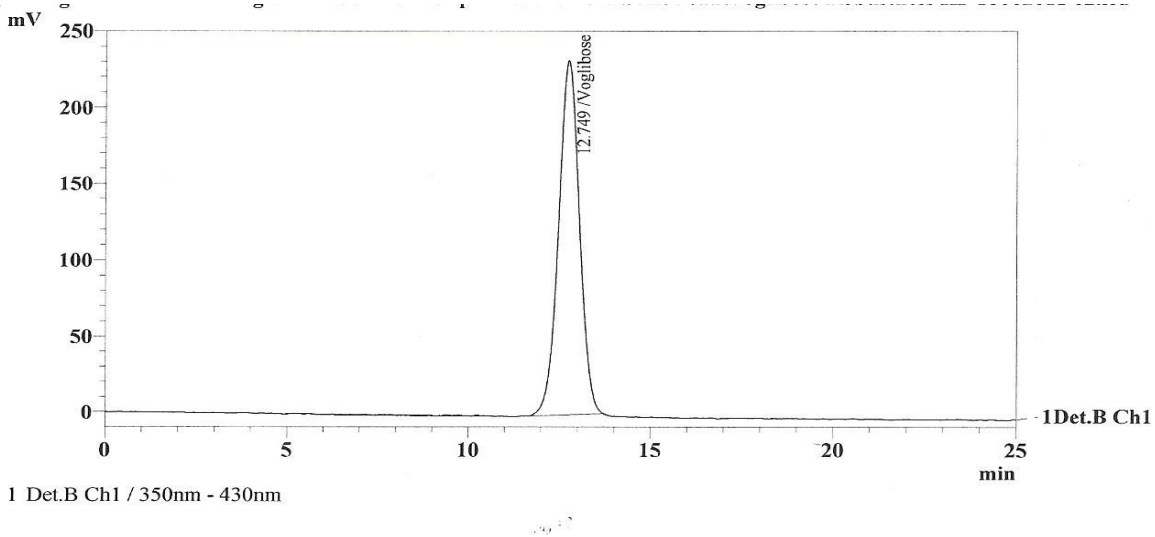


Fig-: Voglibose



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Robustness of High pH (6.7):

Fig-: Metformin HCl

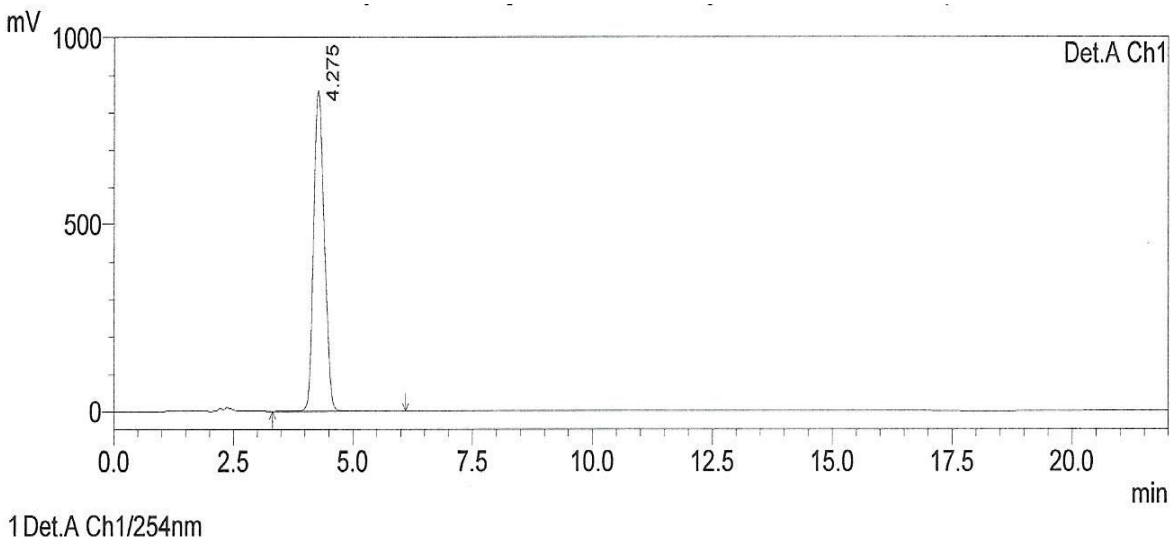

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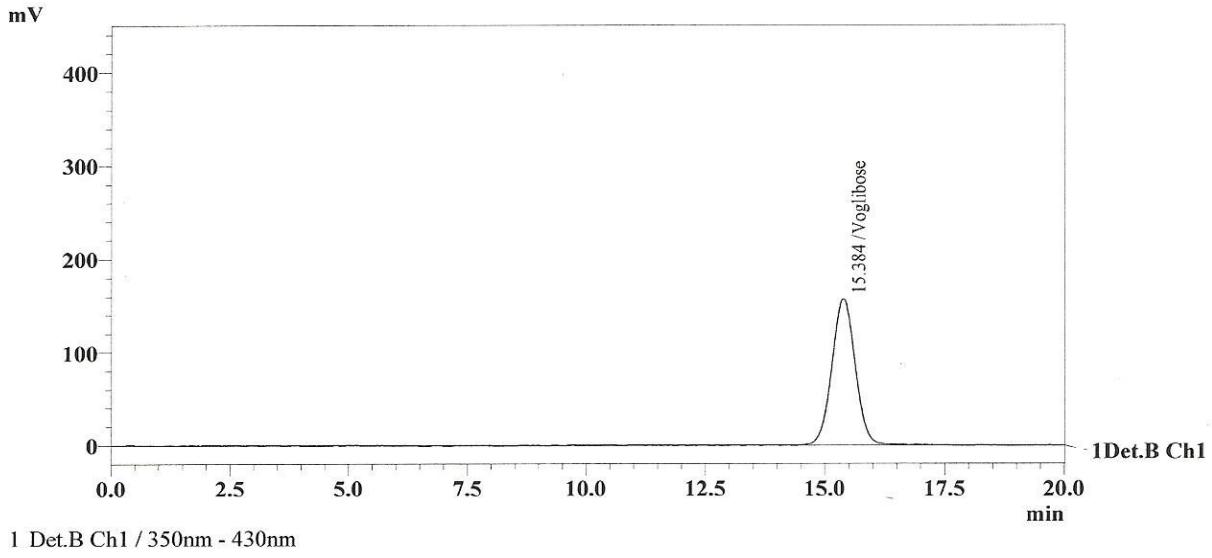
Fig-: Voglibose



Robustness of Low pH (6.3):

Fig-: Metformin HCl

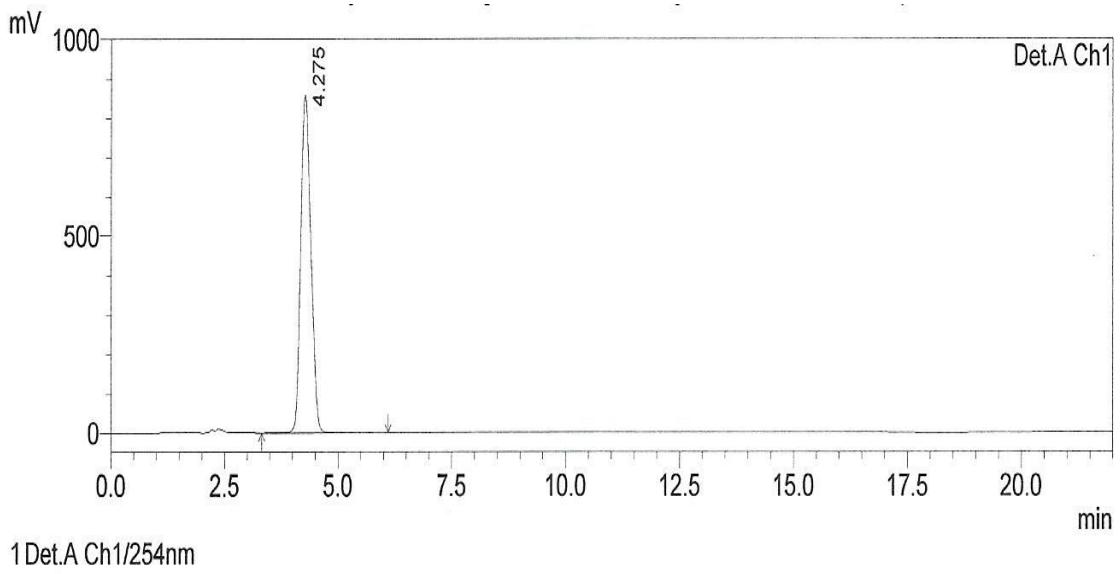
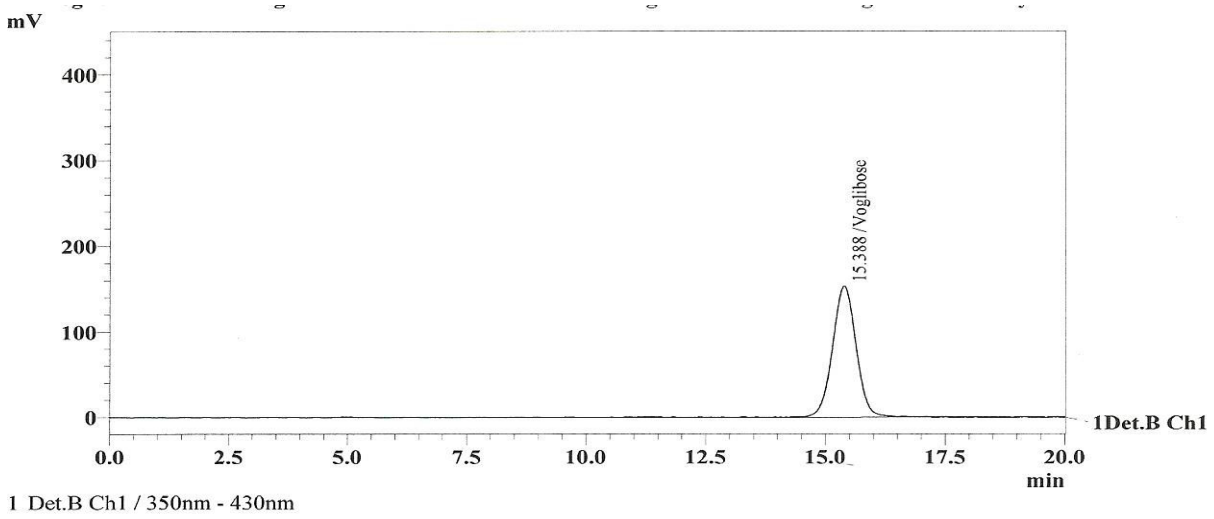


Fig-: Voglibose



RESULTS AND DISCUSSION

Table:I a. System suitability parameters for Metformin HCl

System suitability parameter	**Retention Time**	**AUC**	**Theoretical Plates**	**Tailing factor**
Solution-1	4.27	13441854	5978	1.17
Solution-2	4.25	13569231	5895	1.15
Solution-3	4.50	13442183	5965	1.16
Solution-4	4.31	13395011	5988	1.18
Solution-5	4.55	13454363	5875	1.05
Solution-6	4.51	13460528	5896	1.06
Mean		**13460528.33**		
S.D		**58020.83**		
R.S.D		**0.431**		

Table:I b. System suitability parameters for Voglibose

System suitability parameter	**Retention Time**	**AUC**	**Theoretical Plates**	**Tailing factor**
Solution-1	15.20	119290	5978	1.17
Solution-2	15.10	120153	5895	1.15
Solution-3	15.10	119909	5965	1.16
Solution-4	15.00	120661	5988	1.18
Solution-5	15.12	120353	5875	1.05
Solution-6	15.02	120095	5896	1.06
Mean		**120076.8**		
S.D		**462.8**		
R.S.D		**0.39**		

Acceptance criteria: theoretical plate ≥ 2000, Tailing factor ≤ 2.

Result:

Standard solution of Metformin HCl and Voglibose was determined under proposed condition chromatogram indicating satisfactory % RSD of peak responces,Theoretical plates, tailing factor.

II. SPECIFICITY

As per optimized method Standard and Sample preparations are prepared and injected.

Acceptance criteria: No interference at the Rt of Metformin HCl and voglibose

Result:

No peak observed due to blank, placebo at the retention time of Metformin HCl and Voglibose peak.

III. PRECISION:

Table No :III.a Repeatability

	Metformin HCl		**Voglibose**	
Inj	**AUC**	**Percentage**	**AUC**	**Percentage**
1	13431738	99.7	120983	100.3
2	13422222	99.8	120974	100.4
3	13370026	99.3	120641	100.3
4	13418095	99.7	122999	102.4
5	13434540	99.8	122562	102.0
6	13430087	99.6	122700	102.0
Mean		**99.65**		**101.23**
S.D		**0.19**		**1.00**
R.S.D		**0.19**		**0.99**

Acceptance Criteria: %RSD of the assay percents should not be more than 2. And assay % percent should be 95-105%.

Result:

The relative Standard deviation for the assay of six sample preparations of Metformin HCl and Voglibose was found 0.19% and 0.99%.

CALCULATION:

Amount of Metformin HCl present in mg:

$$\frac{A \times Ws \times 500 \times P \times Aw \times 1000}{B \times 500 \times Wt\ taken \times 100}$$

A = peak area of Metformin HCl for sample preparation

B = peak area of Metformin HCl for standard preparation

Ws = weight of Metformin HCl in mg

P = potency of Metformin HCl

Aw = average weight of the tablets

Amount of Metformin HCl in mg:

$$= \frac{13431738 \times 500 \times 500 \times 100 \times 0.7620 \times 1000}{13460528 \times 500 \times 0.7625 \times 100}$$

= 498.93mg

Assay in % $= \frac{498.93 \times 100}{500}$

= 99.7 %

Amount of Voglibose present in mg:

$$\frac{A \times Ws \times 5x\ 5 \times 500 \times P \times Aw \times 1000}{B \times 100x50x\ 500 \times Wt\ taken \times 100}$$

Assay in % = $\frac{\text{Assay in mg} \times 100}{\text{Label Claim}}$

Amount of Voglibose in mg:

$$\frac{120983 \times 60 \times 5 \times 5 \times 500 \times 99.62 \times 0.7620 \times 1000}{120073 \times 100 \times 50 \times 500 \times 0.7625 \times 100}$$

= 0.3002mg

Assay in % = $\frac{0.3002 \times 100}{0.3}$

= 100.3%

IIIb. Intermediate precision:

Table No:III.b Intermediate Precision

	Metformin HCl		**Voglibose**	
Inj	**AUC**	**Percentage**	**AUC**	**Percentage**
1	13486738	99.5	121125	100.59
2	13555222	100	120065	100.58
3	13480030	99.5	121104	100.66
4	13489595	99.7	123303	102.8
5	13514540	99.8	119562	101.5
6	13575591	100.1	123254	102.3
Mean		**99.77**		**101.41**
S.D		**0.25**		**0.96**
R.S.D		**0.25**		**0.95**

Acceptance criteria: %RSD of the assay percents should not be more than 2. And assay % percent should be 95-105%.

Result:

The difference in the assay of relative standard deviation between two analysts was found to be within the limits only.

CALCULATION:

Amount of Metformin HCl present in mg:

$$= \frac{A \times Ws \times 500 \times P \times Aw \times 1000}{B \times 500 \times Wt\ taken \times 100}$$

Amount of Metformin HCl in mg:

$$= \frac{13486738 \times 500 \times 500 \times 1000.7620 \times 1000}{13460528 \times 500 \times 0.7625 \times 100}$$

= 497.93mg

$$\text{Assay in \%} = \frac{497.93 \times 100}{500}$$

= 99.5%

Amount of Voglibose present in mg:

$$\frac{A \times Ws \times 5 \times 5 \times 500 \times P \times Aw \times 1000}{B \times 100 \times 50 \times 500 \times Wt\ taken \times 100}$$

Assay of Voglibosein mg:

$$= \frac{121125 \times 60 \times 5 \times 5 \times 500 \times 99.62 \times 0.7620 \times 1000}{120073 \times 100 \times 50 \times 500 \times 0.7625 \times 100}$$

= 0.3002mg

$$\text{Assay in \%} = \frac{0.3002 \times 100}{0.3}$$

= 100.3%

IV. LINEARITY:

Table No: IVa. Linearity for Metformin HCl

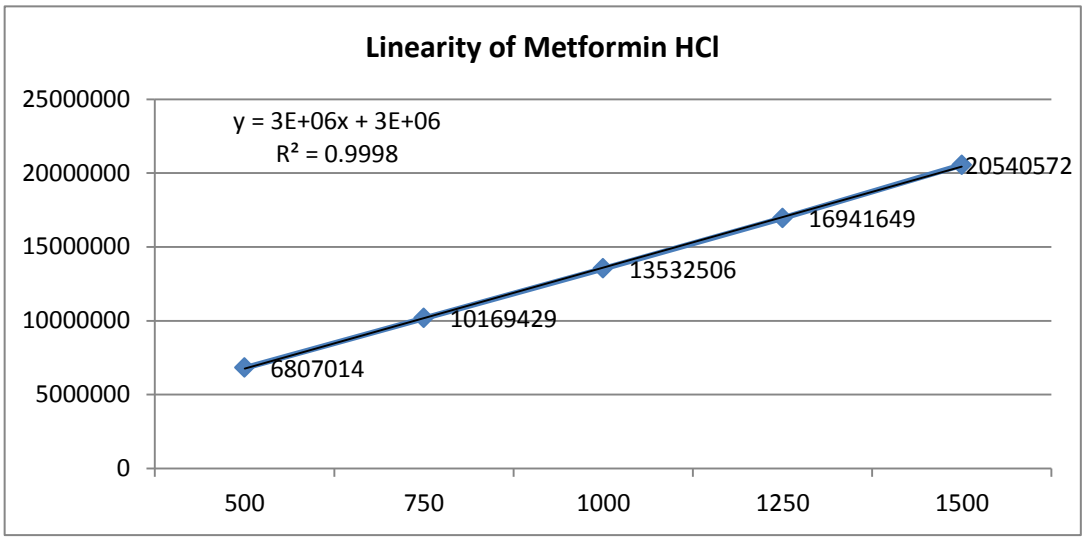
S.No	Test con in%	Con in PPM	Replication inj	Area	Average Area
1	50%	500.00	1	6825366	6807014
			2	6795821	
			3	6799856	
2	75%	750.00	1	10052869	10169429
			2	10256892	
			3	10198527	
3	100%	1000.00	1	13592568	13532506
			2	13499256	
			3	13505695	
4	125%	1250.00	1	16892546	16941649
			2	17012036	
			3	16920365	
5	150%	1500.00	1	20165036	20540572
			2	20935242	
			3	20521439	

Acceptance criteria: r^2 should not be less than 0.99

Result:

The relationship between the concentration and the peak response of Metformin HCl was linear in the specific range and the regression coefficient was found to be 0.999.

Fig No: 1 Linearity curve for Metformin HCl



Calculation for ppm:

Std wt x Pipetted out x 1000

500 x 100

5000 x 5 x 1000 =500 ppm

500 x 100

5000 x 7.5 x 1000 =750 ppm

500 x 100

5000 x 10 x 1000 =750 ppm

500 x 100

5000 x 12.5 x 1000 =750 ppm

500 x 100

5000 x 15 x 1000 =750 ppm

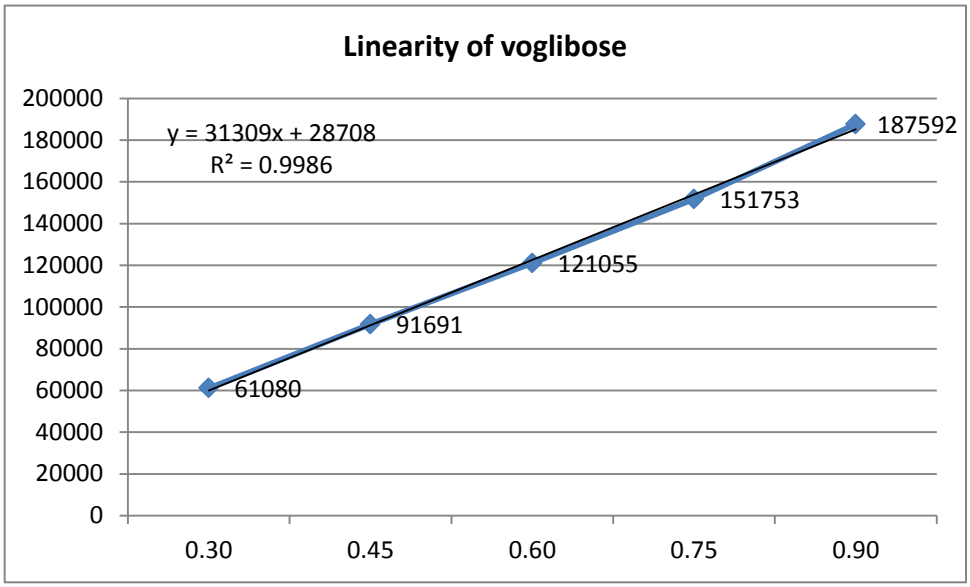
500 x 100

Table No: IVb. Linearity for Voglibose

S.No	Test con in%	Con in PPM	Replication inj	Area	Average Area
1	50%	0.30	1	61259	61080
			2	60958	
			3	61024	
2	75%	0.45	1	91256	91691
			2	92563	
			3	91254	
3	100%	0.60	1	121356	121055
			2	120598	
			3	121210	
4	125%	0.75	1	150569	151419
			2	151236	
			3	152453	

5	150%	0.90	1	186025	187925
			2	187536	
			3	190215	

Fig No: 2 Linearity curve For Voglibose



Acceptance criteria: r^2 should not be less than 0.99.

Result:

The relationship between the concentration and the peak response of Voglibose was linear in the specific range and the regression coefficient was found to be 0.998.

Calculation for PPM:

Std wt x 5 x 5 x 1000

100 x 500 x 100

60 x 5 x 5 x 1000 =0.30ppm

100 x 500 x 100

60 x 5 x 7.5 x 1000 =0.45ppm

100 x 500 x 100

60 x 5 x 10 x 1000 =0.60ppm

100 x 500 x 100

60 x 5 x 12.5 x 1000 =0.75ppm

100 x 500 x 100

60 x 5 x 15 x 1000 =0.90ppm

100 x 500 x 100

Result:

S.NO	**Parameter**	**Metformin HCl**	**Voglibose**
1	Slope	3E	31309
2	Correlation-coefficient	0.999	0.998

V. ACCURACY:

Table No: Va. Recovery data for 50%:

			Metformin HCl		**Voglibose**	
S.NO	**Con in %**	**Amount of Placebo added in (mg)**	**AUC**	**Percentage**	**AUC**	**Percentage**
1	50%	145	6807014	100.6	61080	102.2
2	50%	145	6799013	100.5	61349	102.7
3	50%	145	6805555	100.6	60945	102.0

CALCULATION

Recovery for Metformin HCl in mg:

Sample area x 500 x 500

Std area x 500 x 1

Recovery in % :

Recovery in mg x 100

Wt of Std added

6807014 x 500 x 500 = 2513mg

13550668 x 500 x 1

2513 =50.25%

50

Recovery for Voglibose:

Sample area x 60 x 5 x 500

Std area x 100 x 50 x 1

Recovery in % :

Recovery in mg x 100

Wt of Std added

61080 x 60 x 5 x 500 = 1514mg

120983 x 100 x 50 x 1

Table No: Vb. Recovery data for 75%:

			Metformin HCl		**Voglibose**	
S.NO	**Con in %**	**Amount of Placebo added in (mg)**	**AUC**	**Percentage**	**AUC**	**Percentage**
1	75%	145	10120057	99.7	91691	102.3
2	75%	145	10169429	100.2	91221	101.8
3	75%	145	10233095	100.8	90976	101.5

Table No:Vc. Recovery data for 100%:

			Metformin HCl		Voglibose	
S.NO	**Con in %**	**Amount of Placebo added in (mg)**	**AUC**	**Percentage**	**AUC**	**Percentage**
1	100%	145	13411089	99.1	121055	101.3
2	100%	145	13449139	99.4	122890	102.9
3	100%	145	13532506	100.0	121495	101.7

Table No:Vd. Recovery data for 125%:

			Metformin HCl		Voglibose	
S.NO	**Con in %**	**Amount of Placebo added in (mg)**	**AUC**	**Percentage**	**AUC**	**Percentage**
1	125%	145	16878822	99.8	151419	101.4
2	125%	145	16941649	100.2	151315	101.3
3	125%	145	16836091	99.5	152894	102.4

Table No:Ve. Recovery data for150%:

			Metformin HCl		Voglibose	
S.NO	**Con in %**	**Amount of Placebo added in (mg)**	**AUC**	**Percentage**	**AUC**	**Percentage**
1	150%	145	20540572	101.2	186259	103.9
2	150%	145	20739237	102.2	182972	102.1
3	150%	145	20280016	99.9	183696	102.5

Fig No: 3 Recovery curve for Metformin HCl:

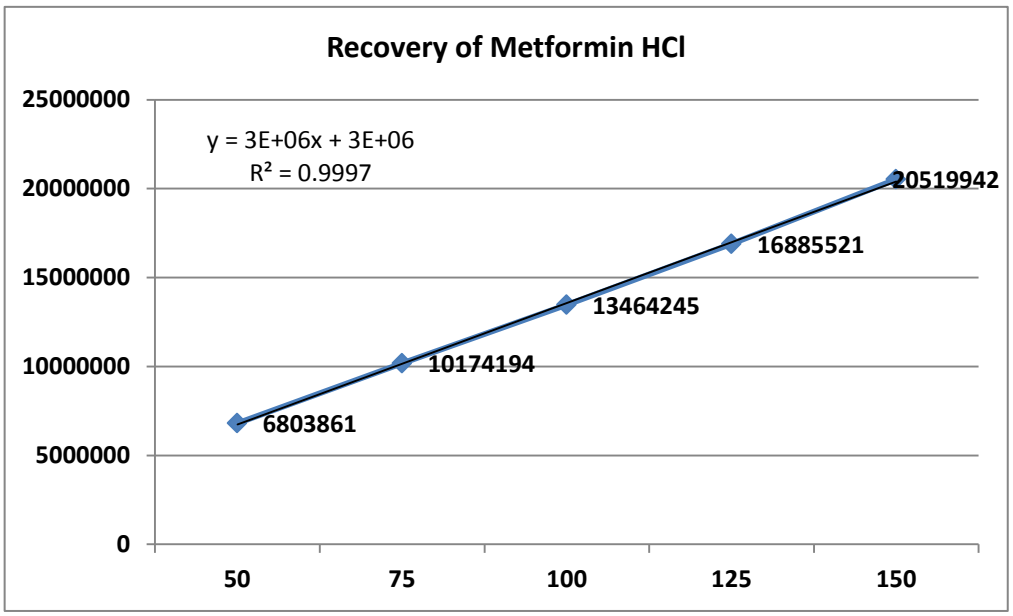


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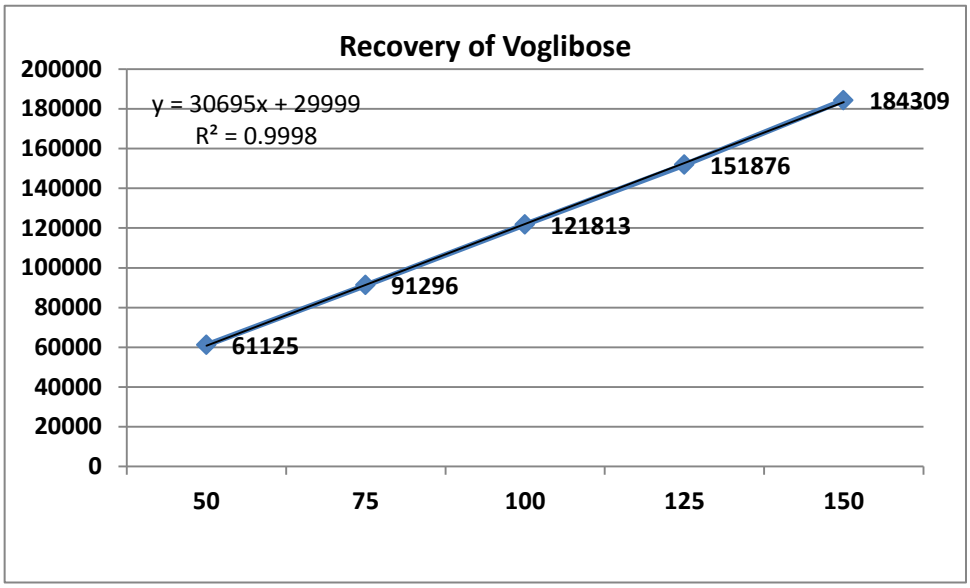
Fig No: 4 Recovery curve for Voglibose



Acceptance criteria: the % recovery of each level should be with in the range of 95-105%

Result:

The percentage of recovery of Metformin HCl and Voglibose was found 97.0% to 103.0%.

VI. SOLUTION STABILITY OF ANALYTICAL SOLUTIONS

Table No:VI. Stability of Analytical solutions

Time interval	**Peak response for Metformin HCl**	**Peak response for Voglibose**
0th hour	13399963	121125
2th hour	13427856	121883
4th hour	13409897	120452
8th hour	13419360	122453
12th hour	13416331	121563

18th hour	13421622	122325
24th hour	13254286	121350
Mean	**13392759.29**	**121593**
S.D	**61709.67638**	**700.0449986**
R.S.D	**0.46076895**	**0.575728042**

Acceptance criteria: %RSD of the peak response should not be more than 2.

Result:

%RSD of Metformin HCl and Voglibose was found 0.46 and 0.57 respectively until 24 hrs.

VII. ROBUSTNESS

Table No: VII a. Robustness High flow(1.2ml):

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13540526	101.7	119025	99.8
2	13302485	99.5	122546	102.3
3	13454865	100.5	120458	100.4
Mean		**100.57**		**100.83**
S.D		**1.10**		**1.31**
R.S.D		**1.10**		**1.29**

Table No: VII b. Robustness Low flow(0.8ml):

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13530522	100.3	122025	100.9
2	13402452	99.7	123006	102.1
3	13334865	99.4	122258	101.7
Mean		**99.80**		**101.57**
S.D		**0.46**		**0.61**
R.S.D		**0.46**		**0.60**

Table No:VII c. Robustness of High Temp(27 C);

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13580524	100.9	121525	101.1
2	13292485	98.6	123046	102.4
3	13334865	99.2	119400	99.4
Mean		**99.57**		**100.97**

S.D		**1.19**		**1.50**
R.S.D		**1.20**		**1.49**

TableNo:VII d.Robustness of Low Temp(23^{O}C):

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13180524	99.0	120025	100.6
2	13092485	98.0	118546	99.0
3	13124865	98.1	118400	98.7
Mean		**98.37**		**99.43**
S.D		**0.55**		**1.02**
R.S.D		**0.56**		**1.03**

Table No:VII e. Robustness of Mobile phase Changes (Buffer:ACN

400:600):

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13680524	102.2	122653	102.5
2	13352485	99.9	121542	101.7
3	13854865	103.2	122412	102.0
Mean		**101.77**		**102.07**
S.D		**1.69**		**0.40**
R.S.D		**1.66**		**0.40**

Table No:VII f. Robustness of Mobile phase Changes (Buffer:ACN 360:640):

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13280524	99.7	121653	102
2	13392485	100.2	117546	98.1
3	13024865	97.3	119412	99.5
Mean		**99.07**		**99.87**
S.D		**1.55**		**1.98**
R.S.D		**1.56**		**1.98**

Table No:VII g. Robustness of High pH(6.7):

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13411089	99.4	121055	101.5
s2	13292485	98.6	123046	102.4
3	13532506	100	122890	102.9
Mean		**99.33**		**102.27**
S.D		**0.70**		**0.71**
R.S.D		**0.71**		**0.69**

Table No:VII h. Robustness of LowpH(6.3):

	Metformin HCl		**Voglibose**	
S.NO	**AUC**	**Assay in %**	**AUC**	**Assay in %**
1	13370026	99.3	120601	100.3
2	13418095	99.7	122999	102.4
3	3434540	99.8	122562	102.0
Mean		**99.60**		**101.57**
S.D		**0.26**		**1.12**
R.S.D		**0.27**		**1.10**

Result:

The assay of Metformin HCl and Voglibose was found 97.0% to 103.0%.

CALCULATION:

Amount of Metformin HCl present in mg:

$$= \frac{A \times Ws \times 500 \times P \times Aw \times 1000}{B \times 500 \times Wt\ taken \times 100}$$

Amount of Metformin HCl in mg:

$$= \frac{13370026 \times 500 \times 500 \times 100 \times 0.7620 \times 1000}{13460528 \times 500 \times 0.7625 \times 100}$$

$$= 496.93mg$$

$$\text{Assay in \%} = \frac{497.93 \times 100}{500}$$

$$= 99.3\%$$

Amount of Voglibose present in mg:

$$\frac{A \times Ws \times 5 \times 5 \times 500 \times P \times Aw \times 1000}{B \times 100 \times 50 \times 500 \times Wt\ taken \times 100}$$

Assay of Voglibosein mg:

$$= \frac{120601 \times 60 \times 5 \times 5 \times 500 \times 99.62 \times 0.7620 \times 1000}{120173 \times 100 \times 50 \times 500 \times 0.7625 \times 100} = 0.3012mg$$

$$\text{Assay in \%} = \frac{0.3002 \times 100}{0.3} = 100.3\%$$

Observation : when flow rate was changed Rt had changed significantly, when mobile phase was changed there was change in the Rt had changed significantly, when the temperature and pH were changed there were no changes observed. So care must be taken while selecting the mobile phase and flow rate.

While changing all the above parameters the assay% was observed to be within limit

SUMMARY

A very few analytical methods appeared in the literature for the determination of Metformin HCl and Voglibose are generally based HPLC,UV, Spectrofluorimetry that has been reported for the quantification of Metformin HCl and Voglibose.

In the present work, an attempt was made to provide a newer, simple, accurate and low cost post column derivatization of spectrophotometric and there derivative method and one HPLC method for the effective quantitative determination of Metformin HCl and Voglibose as an active pharmaceutical ingredient as well as in pharmaceutical preparations without the interferences of other constituent in the formulations.

For routine analytical purposes it is always of interest to establish methods capable of analysing a large number of samples in a short time period with good accuracy and precision. The main purpose of this study was to develop accurate, precise and economic methods for the determination of Metformin HCl and Voglibose. Spectrophotometric technique, and post column derivatization method were applied without using any prior chemical pretreatment in the presence of the strongly overlapping spectra can generate large amounts of data within a short period of analysis.

An HPLC method is developed and validated for various parameters as per ICH guidelines. The system suitability parameters prove that the proposed method is equally suitable for estimation of Metformin HCl and Voglibose, the chromatogram for Metformin HCl and Voglibose were found to be satisfactory on Phenomenex –amino 100A RP-18(2), 250X4.6mm, 5 µm column, using mobile phase composition of Buffer:Acetonitrile [380:620(v/v)] with flow rate of 1.0 ml/min. Both the peaks were found to be symmetrical as found from symmetry factor of 1.01 for Metformin HCl and Voglibose.

The resolution of the proposed method was found to be satisfactory, with peak showing complete base line separation. The retention time for Metformin HCl

about 5 min. and Voglibose was about 13 min. The proposed system of stationary phase and mobile phase was ideally suitable for the estimation as indicated by good number of theoretical plates 5689 per meter for Metformin HCL and Voglibose.

The sensitivity of the method is good and also linearity which is observed good.

The accuracy of method is determined by recovery with spiked concentration of pure drug at three levels for Metformin HCl and Voglibose. The recovery of drug is well within the acceptance limits of 97-103%.

The robust method is as observed from insignificant variation in the results of analysis on changes in mobile phase composition ratio, pH, Flow rate, and Temperature and analysis being performed by different analysts and on different days respectively. In all the above cases the recovery is found to be within the limit.

Result: Chromatographic Condition

Parameters	**Description**
Column	C_{18}:250X4.6mm, 5µ,amino SS Column
Mobile Phase	Buffer:ACN(380:620)
Flow rate	1.0ml/min
Flow rate fluorescence reagent	1.0ml/min
Detection for Metformin HCl	UV-254nm
Detection for Voglibose	Spectrofluorimeter
Excitation wavelength	350nm
Emission wavelength	430nm
Temperature	$25^{O}C$

Injection volume	20µl
Run time	20min
Diluent	Purified water

CONCLUSION

The present study was validated as per the ICH guidelines From the comparative study, it was inferred that the method is simple, specific, precise, linear, sensitive, and also system suitability. The results obtained on the validation parameter met the respective acceptance criteria.

The method was found to have suitable application in routine laboratory analysis and with high degree of accuracy and precision From the comprehensive validation conducted, it was concluded that the method is stable and could be used throughout shelf life of the drug.

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